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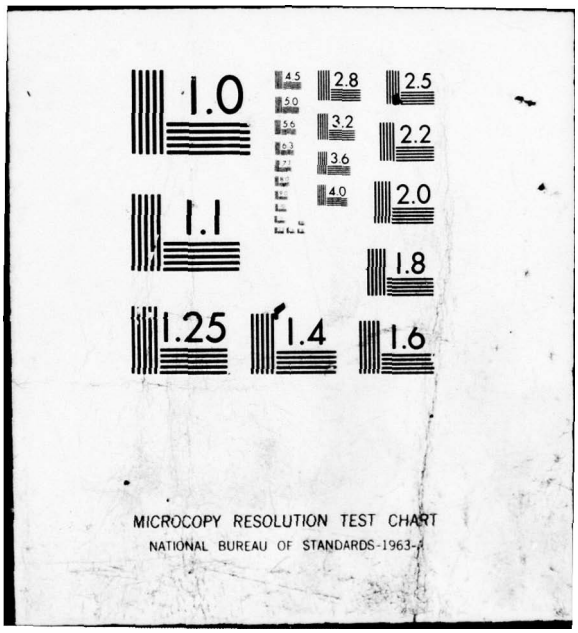
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Studies on the Ecology and Epizoology of the Native Fauna of the Great Salt Lake Desert*

REPORT PERIOD
January 1 to December 31, 1959

ANNUAL SUMMARY PROGRESS REPORT
of the
EXECUTIVE DIRECTOR
and
STAFF

Ecology and Epizoology Series No. 44, June 30, 1960
Ecological and Epizoological Research
University of Utah

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*Supported by U.S. Army Chemical Corps Contract No. DA-42-007-403-CML-427, with the University of Utah

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INTRODUCTION

This report contains the results of studies* on the ecology and epizoology of Q-fever, plague, tularemia, brucella, and Rocky Mountain spotted fever in the wildlife of the southern arm of the Great Salt Lake Desert in northwestern Utah, from January 1 to December 31, 1959. It also contains results of serological studies of blood from domestic animals frequenting this same area.

The overall research objective is to determine, as far as possible, the disease potential of the natural biotic community populations of the study area. Special emphasis is placed on the ecological factors which may play a role in the transmission of the above disease organisms to domestic animals and man. In order to accomplish this objective, the research program is directed toward the study of these diseases in relation to: (1) susceptibility, carrier potential, and bacteremia of each species of animal comprising the populations of the various biotic communities; (2) the potential role of certain ectoparasite populations as vectors; (3) the influence of the ecology of natural community populations on their spread and/or control; (4) their prevalence and incidence in these natural populations; and (5) the chemical control of wildlife ectoparasites.

Supplemental research supporting the major research program includes: (1) colonization and rearing of native animals and ectoparasites; (2) animal behavior; (3) agent virulence, and (4) maintenance of reference collections of the biota studied.

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* Studies by Ecology and Epizoology Research, University of Utah. This project was supported by U. S. Army Chemical Corps Contract No. DA-42-007-403-CML-427.

EXPERIMENTAL Q FEVER INFECTION

1 - IN NATIVE VERTEBRATES

Susceptibility of Six Species of Wild Rodents

Six species of wild rodents, deer mice, Peromyscus maniculatus; pinyon mice, P. truei; meadow mice, Microtus montanus, desert wood rats, Neotoma lepida; Great Basin pocket mice, Perognathus parvus; and western harvest mice, Reithrodontomys megalotis, were found to be highly susceptible to subcutaneous infection with Coxiella burneti, strain AD. However, in all cases the infection was non-fatal and the animals recovered. The diagnosis of infection and the determination of an ID_{50} for each species was based on the presence in the serum of complement fixing antibody titer of 1/64 or greater, 28 days after subcutaneous inoculation of C. burneti. The data are shown in Table 1, with the ID_{50}s and 95% confidence limits calculated by the method of probit analysis (Finney, 1950). The ID_{50}s are in terms of a previously determined 50% infective dose of the frozen C. burneti egg yolk sac slurry in guinea pigs.

TABLE 1. Q Fever Complement Fixing Antibodies in Rodents. Ratio of the number of animals possessing 28-day complement fixing antibody titers of 1/64 or greater to the total number used.

Species	Guinea Pig ID_{50}s of C. burneti injected								Controls		ID_{50}
	10^{10}	10^{8}	10^{6}	10^{4}	10^{3}	10^{2}	10^{1}	1	Ant*	Saline	95% C L
Perognathus parvus Great Basin pocket mouse		8/8	7/7	8/8	6/8		1/7	0/8	1/7	0/10	209 166-263
Reithrodontomys megalotis Western harvest mouse		6/6	6/6	5/5	5/5			0/5		0/6	500 1-1000
Peromyscus maniculatus Deer mouse	16/16	15/16	13/16	15/16	12/16	4/16	0/14	0/16	2/57	0/32	676 123-3715
Peromyscus truei Pinyon mouse				8/8	7/7	4/8	2/9	0/8	0/19	0/14	48 16-148
Neotoma lepida Desert wood rat				8/9	9/9	4/9	1/8	0/9	0/20	0/15	380 60-2399
Microtus montanus Montane meadow mouse	7/7	8/8	8/8	7/8	7/7	3/6	0/8	1/8	0/17	0/18	107 13-871

* Each animal received 0.2 ml of formalized C. burneti antigen

Complement Fixing Antibody Production in Deer Mice

The production of complement fixing (CF) antibody was studied in deer mice after subcutaneous and intraperitoneal inoculation of high (10^7) and low (10^3) guinea pig LD_{50} doses of standard frozen yolk sac slurry of C. burneti. Serum samples were collected at regular intervals for periods up to 10 weeks and titered for CF antibody. An equal number of uninoculated control deer mice maintained in close contact with the infected mice were bled and their sera tested for CF antibody.

Figures 1 and 2 show the development of CF antibody in intraperitoneally and subcutaneously infected deer mice respectively. The effect of dose size on CF antibody production in deer mice was essentially the same whether the mice were inoculated subcutaneoulsy or intraperitoneally. Animals inoculated with the high dose in general produced antibody to higher titer than did mice inoculated with the lower dose. However, the only significant difference in titer appeared between the 16th and 28th day. In this period the animals of both groups possessed high CF antibody titers, but the titers of the low dose groups were significantly lower. Since none of the control deer mice developed significant CF antibody titers, contact transmission of Q fever in this experiment was not demonstrated.

The intraperitoneally inoculated mice were observed for 10 weeks and were found to maintain high CF antibody levels (1/64 to 1/512).

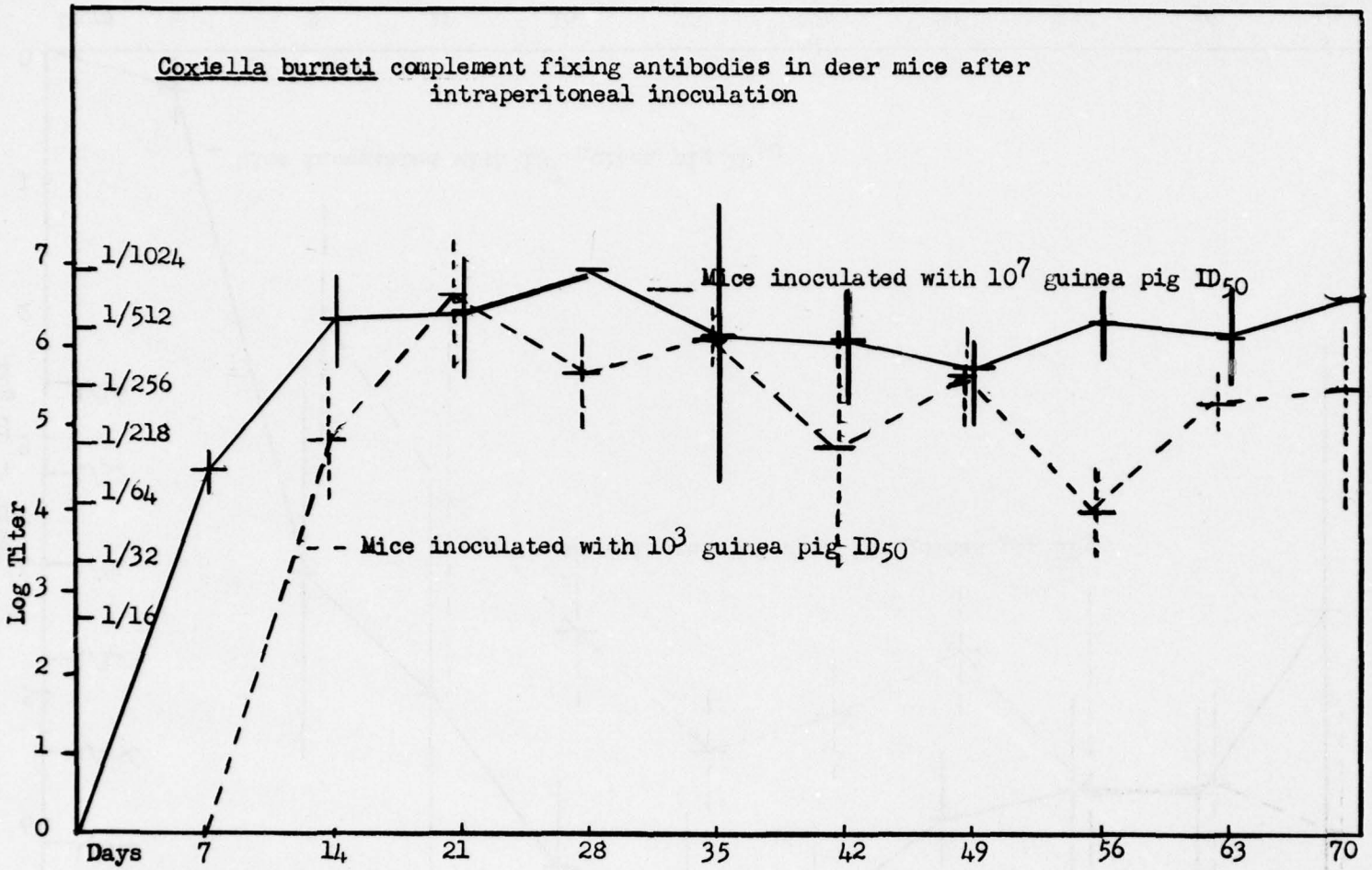

Fig. 1. Development of complement fixing antibody in 2 groups of deer mice inoculated intraperitoneally with 10^7 and 10^3 guinea pig ID_{50} of C. burneti respectively. Mean titers shown by horizontal lines and 95% confidence limits by vertical lines.

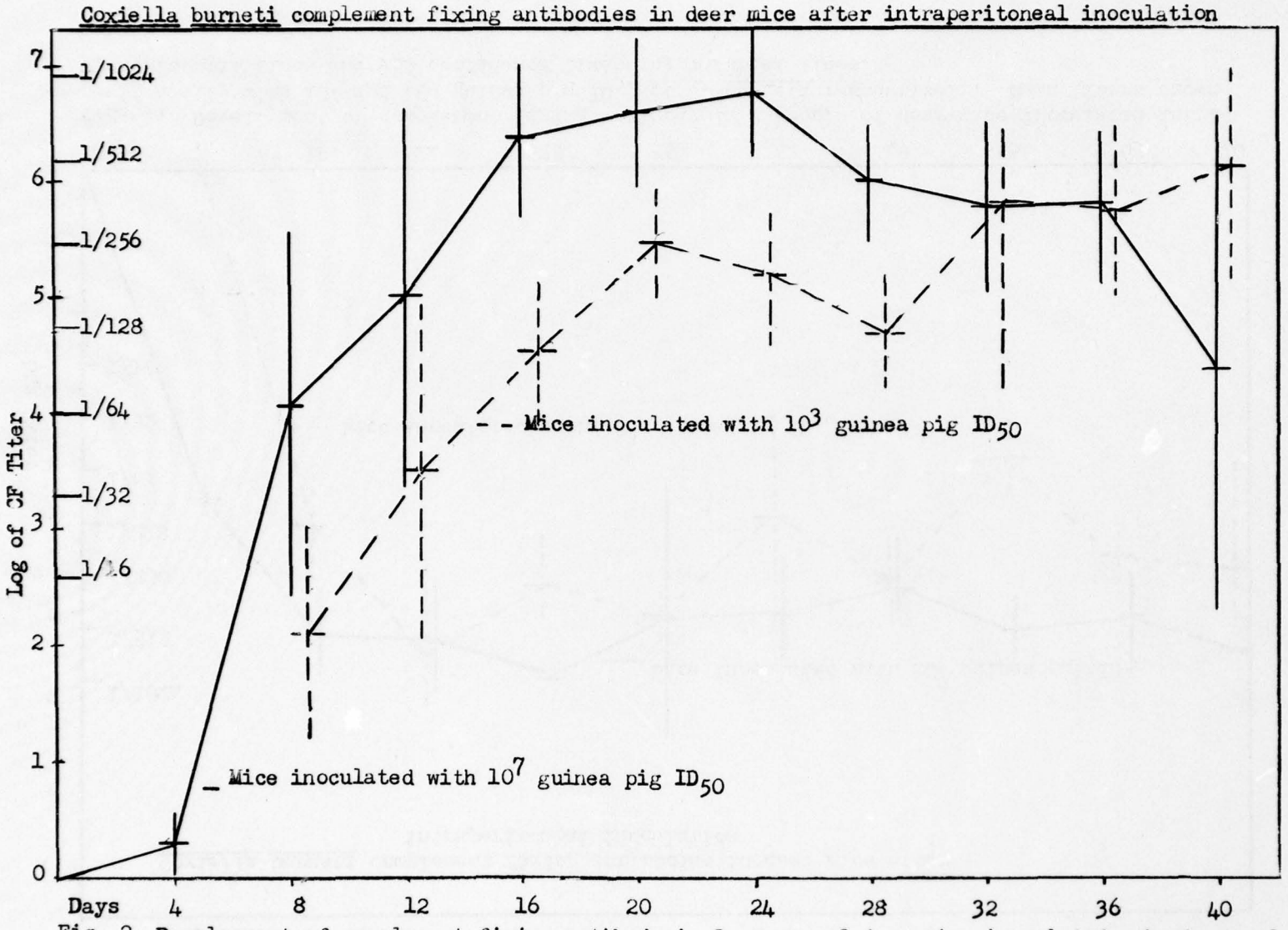

Fig. 2. Development of complement fixing antibody in 2 groups of deer mice inoculated subcutaneously with 10^7 and 10^3 guinea pig subcutaneous ID_{50} of C. burneti respectively. Mean titer shown by horizontal line and 95% confidence limits by vertical line.

Susceptibility of Coyote Pups, Canis latrans lestes, to Subcutaneous and Oral Infection

Fifteen coyote pups approximately 6-8 weeks old were divided into five groups of 3 pups each. A sixth group (VI) had 2 pups. Animals of Groups I and II were inoculated subcutaneously with 10^{10} and 10^{4} guinea pig 50% infective doses of standard C. burneti egg yolk sac slurry.

Group III pups were each fed one guinea pig in the febrile stage of acute Q fever infection. Group IV pups were each fed 5 guinea pigs in the febrile stage of acute Q fever infection over a 2-week period. Group V pups were not exposed to either subcutaneous or oral Q fever infection, but were maintained in the same room with the exposed pups of Groups I to IV. Group VI pups, which served as non-exposed controls, were housed in a separate building free from contact with any Q fever infected animals. Each pup was caged separately and maintained on a diet of freshly killed or frozen guinea pigs.

Approximately 4.5 ml of blood was drawn via the jugular vein of each pup before beginning the experiment and at weekly intervals thereafter. Three ml was saved for collection of serum for CF antibody determinations; the remaining 1.5 ml was inoculated intraperitoneally in 0.5 ml amounts into each of 3 deer mice. These mice were held for 4 weeks, then bled and CF antibody determinations made on the serum samples collected. A CF titer of 1/64 or greater was used to indicate infection with C. burneti caused by rickettsia in the blood of the coyote pup with which the particular mice were inoculated.

Six weeks after final exposure to C. burneti, the coyote pups were killed by exsanguination under nembutal sedation and a post mortem examination performed. Samples of maxillary gland, spleen, liver, mesenteric

lymph gland, kidney, lung, heart muscle, brain, and bone marrow (from the femur) were ground with sterile sand in a mortar and suspended in normal saline. Aliquots of each tissue suspension were inoculated intraperitoneally into each of three deer mice. These mice were held for 28 days, then bled and CF antibody determinations made on the pooled serum samples of each group. The development of a titer of 1:64 or higher was used to indicate Q fever infection caused by rickettsia in the tissue with which the mice had been inoculated.

Appropriate controls were used throughout these experiments.

Coyote pups inoculated subcutaneously with 10^{10} guinea pig ID_{50}s of C. burneti (Group I) showed no overt signs of infection. but responded by rapid production of complement fixing antibody, Table 2.. The antibody titer was maintained at a high level throughout the 6-week period.

Pups inoculated with 10^{4} guinea pig ID_{50} doses developed an erratic antibody response, probably indicating infection of a low and fleeting nature (Group II).

Pups exposed to oral infection developed either low or no CF antibody (Groups III and IV), indicating a high degree of resistance to Q fever via this route. The non-exposed control pups failed to develop CF antibody titers during the course of this experiment.

Rickettsia were demonstrated in the blood of all 3 coyotes of Group I, one week after inoculation with 10^{10} guinea pig ID_{50}, and in one of these pups at 2 and 3 weeks as well. None of the tissues of any of the 15 coyote pups contained C. burneti at autopsy 6 weeks after the last exposure to Q fever infection, Table 3.

TABLE 2. Coxiella burneti Complement Fixing Antibodies in Coyotes. Complement fixing antibody titers in coyote pup sera after exposure to C. burneti via the oral and subcutaneous routes. Data are shown as the reciprocal of the highest serum dilution causing 50% red blood cell lysis. (-) indicates a titer of less than 1/64.

Weeks post Exposure	Group I			Group II			Group III		
	1	2	3	1	2	3	1	2	3
0	-	-	-	-	-	-	-	-	-
1	512	512	-	-	-	-	-	-	64
2	1024	1024	512	256	256	-	-	256	-
3	4096	256	128	-	64	-	-	-	-
4	512	256	256	AC*	-	-	-	-	-
5	512	128	512	-	-	128	-	-	-
6	1024	256	128	64	128		64	-	-

* AC - Serum anticomplementary

Group I. Three pups inoculated subcutaneously with 10^{10} guinea pig ID_{50}.
Group II. Three pups inoculated subcutaneously with 10^{4} guinea pig ID_{50}.
Group III. Three pups each fed one infective guinea pig.

TABLE 2 (continued)

Weeks post Exposure	Group IV			Group V			Group VI	
	1	2	3	1	2	3	1	2
0	-	-	-	-	-	-	-	-
1	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-
3	64	-	-	-	-	-	-	-
4	-	-	-	-	64	-	-	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	64	-	-
7	-	-	-	-	-	-	-	-
8	64	-	-	64	-	-	-	-

Group IV. Three pups each fed a total of 5 infective guinea pigs over a 2-week period.
Group V. Three pups unexposed, but kept in the same room as exposed pups.
Group VI. Two pups unexposed, and kept in a separate building.

TABLE 3. Rickettsemia in Coyotes. Rickettsemia in coyote pups exposed to subcutaneous and oral Q Fever Infection.

Weeks post Exposure	Group I *			Group II			Group III			Group IV			Group V			Group VI	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2
0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1	/	/	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	/	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* See Table 2 for description of groups.

A more detailed study of the pathogenesis of C. burneti in coyote pups exposed to oral and subcutaneous infection was conducted, using 5 animals. Three were inoculated subcutaneously with approximately 10^{10} guinea pig ID_{50}s of C. burneti yolk sac slurry, and two were each fed two Q fever infected guinea pigs in the febrile stage of the disease. Each animal was bled at 2-5 day intervals for determination of CF antibody and rickettsemia as described above. One of these subcutaneously inoculated pups was killed at the end of 1 week, one at the end of 2 weeks, and one at the end of 3 weeks, respectively. One of the pups fed infected guinea pigs was killed at the end of 2 weeks and one at the end of 3 weeks. Each pup was autopsied and the various tissues assayed for the presence of rickettsia as described above.

In two of the three subcutaneously inoculated pups, rickettsia were demonstrated in the blood stream at 2, 4 and 7 days after inoculation, respectively, Table 4. In the third pup (No. 2), no rickettsia were isolated from the blood. It is of interest that this latter pup had a pre-infection titer of 1/128, indicating previous exposure to Q fever and a certain degree of immunity, Table 5.

Rickettsia were demonstrated in the blood of one of the two pups 2 days after oral infection, but at no other time, Table 4.

Rickettsia were demonstrated in the maxillary gland, liver and spleen of the subcutaneously inoculated pups killed 7 days after inoculation. All tissues of the pup killed on the 14th day after inoculation were sterile. As previously noted, this animal had a pre-inoculation titer, indicating some immunity to infection.

Neither of the two pups exposed to oral infection possessed rickettsia in their tissues 14 and 21 days after exposure, respectively.

TABLE 4. Rickettsemia in Coyotes. Development and persistence of rickettsemia in coyote pups experimentally infected with C. burneti via the subcutaneous and oral routes.

Days post Exposure	Subcutaneous Inoculation			Oral Infection	
	No. 1	No. 2	No. 3	No. 1	No. 2
0	-	-	-	-	-
2	≠	-	≠	-	≠
4	≠	-	≠	-	-
7	≠	-	-	-	-
9		-	-	-	-
11		-	-	-	-
14		-	-	-	-
16				-	-
21				-	-

TABLE 5. Antibody Formation in Coyotes. Development of CF antibody in coyote pups experimentally infected with C. burneti via the subcutaneous and oral routes. Data presented as the reciprocal of the highest serum dilution that caused 50% lysis of sensitized sheep red blood cells. Only titers of 1/64 or higher are considered to be significant.

Days after Exposure	Subcutaneous Inoculation			Oral Infection	
	No. 1	No. 2	No. 3	No. 1	No. 2
0	-	128	-	-	-
7	64	64	>256	-	64
14		128	256	64	64
21			>256		64

Susceptibility of Adult Bob-Cats, Lynx rufus

Two adult bob-cats, each fed three Q fever infected guinea pigs in the febrile stage of illness, were bled prior to infection and weekly thereafter for CF antibody determination and rickettsemia analysis. A third bob-cat was not exposed to Q fever infection and was maintained in a separate building as a control.

Only one of the two bob cats fed infected guinea pigs developed a mild CF antibody response. In this animal, antibody was present by the third week and persisted through the sixth week when the animal was killed. The other two bob cats failed to develop any CF antibody titer, Table 6.

The bob cat that developed the titer had rickettsiae in its blood stream one week after exposure to oral infection, but not thereafter. Neither of the other two cats developed a rickettsemia.

The tissues of all these bob-cats were sterile when tested six weeks after the experiment began.

Another bob-cat inoculated subcutaneously with approximately 10^{10} guinea pig ID_{50} of C. burneti was bled at 2-4 day intervals for 15 days and the blood tested for rickettsia as previously described. Rickettsia were isolated from the blood of this animal at 2, 4, 7, and 9 days after inoculation, but not at 11 and 15 days. On the 15th day the CF antibody titer had risen from 0 to 1/64.

At autopsy on the 15th day, the only tissue containing viable C. burneti was the spleen.

TABLE 6. Antibody Titers in Bob Cats. CF antibody production in bob cats exposed to oral Q-fever infection. Titers expressed as the reciprocal of the highest serum dilution causing 50% lysis of sensitized sheep red blood cells. (-)= titer of less than 1/64.

Weeks post Exposure	Bob Cat No. 1	Bob Cat No. 2	Bob Cat No. 3
0	-	-	-
1	-	-	-
2	-	-	-
3	128	-	-
4	128	-	-
5	128	-	-
6	64	-	-

Susceptibility of the Red-tailed Hawk, Buteo jamaicensis calurus to Oral Infections

A red-tailed hawk fed six Q fever infected deer mice failed to develop any signs of infection. There was no rise in CF antibody titer over a 28-day period. All tissues examined 28 days after exposure to infection were sterile.

2 - IN NATIVE FLEAS

Techniques Employed

Flea samples were placed in white glazed porcelain drop slides containing .1 ml of sterile saline. Each sample was then triturated by grinding with the bottom surface of an abraded pyrex serum tube (7x75 mm). After grinding, the triturate was diluted with another .25 ml of sterile saline containing $1:10^{10}$ parts penicillin and inoculated into the yolk sacs of five (5 to 7-day old) embryonated hen's eggs. The eggs were candled daily, and after 10 days yolk sacs from each group of 5 eggs were ground in Ten Broeck tissue grinders diluted with 1 ml of sterile saline and in-

oculated into 5 new eggs. Slides were prepared and stained with Machiavello's stain from each successive yolk sac transfer, and examined for the presence of rickettsiae.

Flea triturates were also inoculated into healthy deer mice and guinea pigs. Only part of the flea samples were tested by this method, and of those none of the test animals survived in our laboratory after an air conditioning system failure on about the 10th day following the challenges.

Fleas used in these experiments were obtained from our colony and in most cases were young adults. In all cases they had been without feeding 24-48 hours prior to an infected blood meal. In some experiments fleas were placed with an infected host for only short (20-30 minute) periods, in others for periods as long as 48 hours. Samples were examined microscopically to determine whether or not fleas had fed.

Effect of Temperature on Flea Longevity

In order to determine flea longevity after removal from infected hosts, part of the fleas were stored at room temperature (17-27°C), and part at 4°C. Optimum storage temperature for fleas used in these experiments has not been determined. However, fleas stored at the high temperatures had a high death rate over a very short period (2-3 days), whereas fleas stored at the lower temperature were maintained for several weeks. It seems probable that the lower temperatures during winter months may influence disease persistence in these ectoparasites. Much work is needed to solve this problem. Special work is required in order to determine the effects of temperature on Q fever organisms in the ventriculi of fleas, and also the disposition of the organisms after entry into the digestive tract of the flea.

A related experiment to determine the longevity of normal fleas of the species Orchopeas leucopus, at various temperature ranges was initiated. Four groups of 50 fleas per group were held in procaine vials sealed at one end with rubber stoppers, and sealed at the other end by cotton plugs. Temperatures investigated were (a) 5.6°C, (b) 18°C, (c) 37°C, and (d) 20-28°C. The resultant longevity figures of (a) and (b) were 42 days, while those held at the higher temperatures, (c) and (d), died within a 3 and 5-day period. Since fleas were unable to feed in this experiment, dessication and/or starvation seemed to be the major factor in their death. Fleas from groups (c) and (d) were cleared of evidence of their last blood meal, whereas fleas from groups (a) and (b) had remnants of this meal visible up to 20 days after feeding.

Infection in Two Species of Fleas

Eleven tests were designed and conducted to determine whether or not Coxiella burneti could be isolated from fleas after feeding on infected hosts. These tests also indicated longevity of viable organisms in fleas following infective blood meals.

Three experiments were initiated to determine the extant to which individual fleas of the species O. leucopus could transmit Q fever rickettsia from infected pinyon mice to healthy deer mice (naked strain). Fleas were placed on the host animals for only short periods of time daily over a 5-day period.

Seven experiments were conducted to determine if groups of 25 to 75 fleas could transmit C. burneti when allowed to remain in a nest with healthy pinyon mice for extended periods of time.

Since in all of the above testing, techniques were of necessity developed and improved subsequent to the progress of the experiment, results obtained are far from complete. However, the above experiments have contributed information that has been utilized to develop techniques for improved experimentation on those problems especially concerning the isolation of C. burneti from fleas. Two tables (7 and 8), show the results of these studies.

Table 7 shows the results of Q fever infection and transmission rates of 1,225 fleas of two species, O. leucopus and Thrassis bacchi gladiolis. Samples of 78 fleas, in groups of 5 to 12 fleas each, were tested for the rickettsia. Of these, six (7.6%) were found to be positive.

From the remaining fleas, 84 individual transmissions were attempted, using one flea per healthy animal and 7 groups of 25 fleas per group per host. There were no positive results. It is possible some infections may have been undetected since it is extremely difficult to determine rickettsial organisms taken from egg yolk cultures having fewer than $1x10^6$ organisms per ml of substrate, and it is further probable that fleas having a low concentration of organisms were overlooked and determined to be negative. Part of the above mentioned tests were further determined by guinea pig and deer mouse inoculation to establish presence of rickettsial infection in fleas. The larger part of the experimental animals challenged in this manner were killed approximately 11 days after inoculation by excessive heat in the laboratory. However, none of the animals showed a clinical response to the flea inocula which would indicate Q fever infection. Of those which were not killed, sera samples were taken and stored for future serological tests.

Q Fever Longevity in Fleas

Table 8 shows longevity results of Q fever infection obtained from an experiment in which groups of 12 fleas were tested individually at weekly intervals for C. burneti. Positive samples were obtained only during the first two weeks of testing.

TABLE 7. Transmissions. Q fever infection and transmission in two species of native fleas.

Host Species	Flea Species	Total used	Infected/ tested	Transmissions animal
Peromyscus maniculatus	Orchopeas leucopus	350	1/15	0/50
P. maniculatus	Thrassis bacchi gladiolis	250	1/10	0/55
P. truei	O. leucopus	525	4/48	0/105
P. truei	T. b. gladiolis	100	0/5	0/24
	Totals	1,225	6/78	0/234

TABLE 8. Longevity. Longevity of Coxiella burneti in Orchopeas leucopus fleas. *

Host Species	Weeks after infection				
	1	2	3	4	5
Peromyscus truei	1/12	3/12	0/12	0/12	0/12
	2/10	1/12	0/12		
P. maniculatus (naked strain)	0/5	2/12			

* Tested by egg yolk sac slide preparation following first, second and third passages in eggs.

3 - IN NATIVE TICKS

Work Loss

Approximately one year's work comprising numerous experiments with native ticks was disrupted during the current report period, by death of the vectors and hosts due to excessive heat, resulting from a mechanical breakdown in the cooling system. Some of the lost vectors had been holding virulent tularemia organisms in excess of two years. Others had been just infected with plague and Q fever organisms. In some cases the entire stock of certain species was lost.

Although the following results are incomplete because of this loss, an attempt has been made to report the results at the time of death of the ectoparasites. In some cases infection was evidenced but not confirmed. In others, the ticks were at, or too near, critical stages in their life cycle to enable laboratory infections. In still others, the entire basic rearing stock was lost. Nevertheless, considerable information is presented in these necessarily incomplete results.

Ixodes kingi

In an experiment to test for ovarian transmission of Q fever by this species of tick, adult females were fed on Coxiella burneti-infected kangaroo rats. Larvae hatched from the eggs, but were killed by excessive heat in the laboratory before they could be tested for the presence of the agent.

Dermacentor parumapertus

In two experiments to test transmission of Q fever by this species of tick, an attempt was made to feed larvae on C. burneti-infected deer mice, but the larvae failed to attach. In 8 other experiments, kangaroo

rats were substituted as infected hosts. Approximately 300 fed larvae were recovered from Q fever infected kangaroo rats. Ten pools of 2 ticks each were triturated and inoculated into 2 guinea pigs each. Guinea pigs from 8 of these pools developed temperatures in excess of 103°F. Excessive heat in the laboratory killed the guinea pigs before they could be tested serologically for antibodies against Q fever. The ticks were also killed before they were tested for transmission of the agent to healthy animals.

In another experiment, approximately 50-100 nymphs of this tick were placed on each of 4 C.burneti-infected kangaroo rats. Seventy-four engorged nymphs were recovered. Six of these were triturated in saline and inoculated into 2 guinea pigs each. One or both guinea pigs from each pair developed temperatures in excess of 103°F, but died of excessive heat in the laboratory before serological tests could be conducted. The ticks also died, after 59 had molted to adults.

Ornithodoros hermsi

Three of 4 adult ticks of this species in one experiment, and one of 4 in another, fed to repletion on C. burneti-infected guinea pigs. Because of scarcity of these specimens, none of these ticks were tested for infection before they were killed by excessive heat in the laboratory.

In another experiment, 40 larvae were placed on a C. burneti-infected wood rat, Neotoma lepida, of which 31 fed. A sample of three of these were tested individually by inoculation into two guinea pigs each. All pigs developed temperatures between 104° and 105°F. One pig from each pair was sacrificed and spleen impressions showed Q fever organisms. The remaining pigs and ticks were killed by high temperatures in the laboratory.

Ornithodoros parkeri

In three experiments with this species of ticks 10, 8 and 12 nymphs respectively were successfully fed on C. burneti-infected guinea pigs. Two ticks from the last group were triturated individually and inoculated into 2 guinea pigs each. One pig from each pair developed a temperature in excess of 103°F, but all 4 pigs died of excessive heat before the organism could be recovered. The ticks also died before they could be fed on healthy animals for possible transmission of the organism.

Otobius lagophilus

Of approximately 2,000 larvae of this species of tick which were placed on a C. burneti-infected jack rabbit, 105 were recovered as fed nymphs. The host rabbit died, apparently of an organism other than C. burneti. An organism was isolated from spleen tissue but was lost before identification was completed. Samples of the remaining ticks indicated they apparently were infected with the same organism, but the ticks were killed in the laboratory by excessive heat, and the organism could not be re-isolated.

EXPERIMENTAL PLAGUE INFECTION

1 - IN NATIVE MAMMALS

Susceptibility of Native Rodents to Experimental Plague Infection

Experimental infection of meadow mice, Microtus montanus, with Pasteurella pestis (Alexander strain), subcutaneously, elicited a very heterogeneous response similar to that previously described in deer mice. An LD_{50} of 4.3×10^2 organisms was obtained, but the 95% confidence limits were 8 to 4.2×10^3 organisms.

Bushy-tailed wood rats, Neotoma cinerea, were resistant to a dose as high as 10^3 organisms inoculated subcutaneously. Lack of a sufficient number of animals prevented the establishment of an LD_{50} for this species.

Characterization of Two Enzootic Strains of P. pestis

Two strains of P. pestis isolated from fleas (DPG-1) and a kangaroo rat (DPG-2), were tested for virulence for several species of animals, Tables 9 and 10. The characterization of these strains has not been completed as of this writing, but the preliminary data indicate that both strains are perhaps slightly less virulent than the Alexander strain, Fig. 3. This was especially apparent in deer mice inoculated intraperitoneally. There was no difference in virulence of the 3 strains when assayed by subcutaneous inoculation into deer mice.

There was some indication that strain DPG-1 is more virulent than DPG-2 for white mice and deer mice (I.P.). However, conclusive evidence is still lacking.

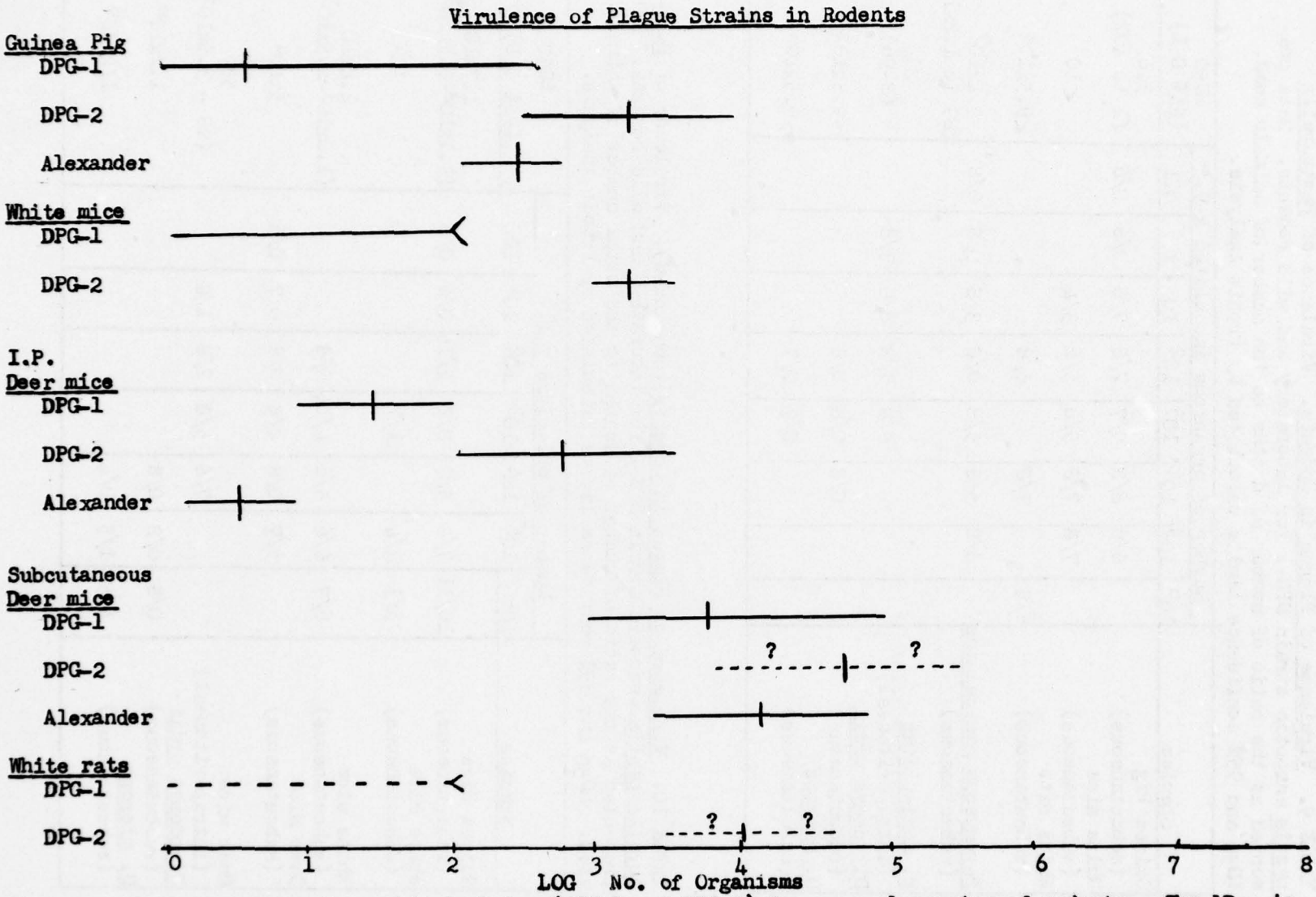

Fig. 3. Virulence of two P. pestis isolates (DPG-1 and DPG-2) for several species of rodents. The LD_{50} is represented by the vertical line, the 95% confidence limits by the horizontal line. The results of virulence titration of the Alexander strain in guinea pigs and deer mice are presented for comparison.

TABLE 9. Virulence of Plague in Rodents. Virulence of Pasteurella pestis enzootic strain DPG-1 for laboratory and wild rodents. Data presented as the ratio of number of deaths to the number of animals used. LD_{50} and 95% confidence limits calculated by Probit Analysis.

Species	Number of organisms inoculated x 9.5								LD50 (95% C L)
	10^6	10^5	10^4	10^3	10^2	10	1	0.1	
Guinea Pig (subcutaneous)		6/6	8/8	5/7	7/8	7/6	0/6	0/6	3.6 (1 to 407)
White Mice (subcutaneous)		7/8	7/8	5/6	5/6	3/4			<10
White rats (subcutaneous)	8/8		7/8		6/8				$<9.5x10^2$
Peromyscus maniculatus (subcutaneous)		8/8	5/8	5/8	0/8	3/8	0/8	0/8	$9.4x10^3$ (89 to $1.2x10^4$)
P. maniculatus (intraperitoneal)				8/8	7/8	8/8	3/8		28 (8-96)
Dipodomys ordii (subcutaneous)			0/8	0/8	0/8				$>9.5x10^5$
D. microps (subcutaneous)				0/7	0/7				$>9.5x10^4$

TABLE 10. Virulence of Plague in Rodents (continued). Virulence of Pasteurella pestis enzootic strain DPG-2 for laboratory and wild rodents. Data presented as the ratio of number of deaths to the total number of animals used. LD_{50} and 95% confidence limits calculated by Probit Analysis.

Species	Number of organisms x 1.2							LD50 (95% C L)
	10^6	10^5	10^4	10^3	10^2	10^1	10	
Guinea Pigs (subcutaneous)	10/11	7/8	5/8	8/8	2/14	0/6	0/6	$1.7x10$ ($3.1x10^2$-$8.9x10^3$)
White rats (subcutaneous)	3/3	4/4		1/7				10^4
White mice (subcutaneous)	6/7	6/6	5/6	4/11	0/8			$2.4x10$ ($1.0x10^3$-$4.5x10^3$)
Deer mice (subcutaneous)		6/7	0/8	0/8	0/8	0/7	0/6	$5x10^4$
Deer mice (intraperitoneal)			7/8	5/8	2/8	1/8		589 (98 - $3.5x10^3$)
Dipodomys ordii (subcutaneous)	0/8	0/8	0/8					$1.2x10^6$
D. microps (subcutaenous)	1/5	1/5	0/4					$1/2x10^6$

2 - IN NATIVE FLEAS

Orchopeas sexdentatus

A number of transmission experiments with this species reported in the 1957-58 Annual Report of this program showed no positive plague transmission after testing a total of 1,700 fleas, either in groups or in tests using individuals. Studies with this vector were continued during this report period. Three experiments were conducted in which 25 fleas per experiment were fed individually. In these 7 (70%) of a sample of 10 fleas exhibited plague organisms after feeding on infected pinyon mice hosts. In subsequent experiments, none of these fleas transmitted plague to healthy pinyon mice or deer mice.

In another experiment designed to test the serial transmission ability of infected fleas, a group of 70 fleas were fed on infected deer mice. They were placed on a different host daily for a period of 10 days beginning after the first evidence of blockage in the flea. The fleas acquired unrestricted feeding over the 10-day period prior to proventricular blockage. On the 6th day samples from 16 fleas were tested individually for plague organisms. Results showed that 56% were infected. One transmission occurred on the 7th day after the serial feeding was initiated. This was the first and only plague transmission recorded for this species in this laboratory, Table 11. It is interesting to note that even though each flea had fed on several healthy host animals, the one which transmitted, transmitted to only one of the hosts at a specific time.

Monopsyllus w. wagneri

Since the colony of this species is relatively new in this laboratory and the numbers produced are few, only three transmission experiments with individuals were conducted.

In each of these 3 experiments, 24 fleas were exposed to *P. pestis* infection by feeding on infected pinyon mice. Samples of each group of fleas showed 66% infection after feeding. In one experiment, a single flea transmitted the disease organism; in another, there were two transmissions; and in the third, none. These experimental results indicated that *M. wagneri* is a capable plague vector in this area. In general, this species is abundant on deer mice throughout the local biotic communities, occurring in juniper brush and shadscale-gray molly-greasewood most frequently during spring and fall months. From all indications it occurs in other communities during the same periods, and probably follows the same seasonal pattern of activity. Interestingly enough, this species has been known to infest hosts other than deer mice quite frequently and is collected along with several different species of fleas on deer mice during all seasons of the year. Since the deer mouse is the most widespread rodent species in the study area, this flea may be important epizoologically.

TABLE 11. Plague Infection in Native Fleas

Host	Flea Species	No.	Infected/ tested	Transmissions/used	
				individual	group
Peromyscus truei	*Monopsyllus w. wagneri*	120	5/8	1/24	-
Onychomys leucogaster	*M. w. wagneri*	80	6/8	0/24	-
P. maniculatus	*M. w. wagneri*	200	5/8	2/24	-
P. maniculatus	*Orchopeas sexdentatus*	100	7/10	0/75	-
P. truei	*O. sexdentatus*	100	9/16	0/15	1/70

3 - IN NATIVE TICKS

Ornithodoros hermsi

Fifty-eight first instar nymphs of this tick species were placed on a guinea pig dying of P. pestis infection. Seven failed to show any sign of a blood meal, 12 partially fed, and 39 fed more or less to repletion. The results of this experiment will be given in a subsequent report.

TULAREMIA INFECTION IN NATIVE TICKS

Otobius lagophilus

An experiment with this species of tick which was begun September 12, 1957, was culminated in August 1959, when excessive heat killed the remaining ticks. A total of 262 fed nymphs had been recovered from two jack rabbits which had died of tularemia October 13, 1957. Samples of these ticks were tested for the presence of P. tularensis on Nov. 14, 1957; January 6, July 2, and October 2, 1958; January 17, June 24, and August 20, 1959. All mice inoculated with ticks from this experiment died of tularemia. This experiment also indicated that P. tularensis infection had an adverse effect on ticks. Of the 262 nymphs harvested, 53 (21%) failed to molt to adults. Of 109 uninfected ticks used as controls, only 12 (11%) did not molt. Of the 103 infected females that molted, only 61 (59%) laid eggs. Of the 67 control females that molted, 56 (85%) laid eggs. Although no counts were made, visual appraisal from clutch sizes indicated that the infected females laid not more than half as many eggs per tick as the controls.

In order to test ovarian transmission, two pools of 200 eggs each from 15 and 12 infected females were inoculated into healthy deer mice with negative results. A pool of 200 larval ticks hatched from eggs from these infected females did not transmit tularemia when fed on a susceptible jack rabbit.

Other adult ticks from this experiment were placed in the cage litter of two Ord and two chisel-toothed kangaroo rats, resulting in transmission of the disease to all of the rats. Since the adult ticks of this species do not feed, the most plausible explanation of the infection is through ingestion of the tick by the rats. Inasmuch as both species of kangaroo rats are common hosts to the immature stages of the rabbit tick Dermacentor parumapertus, and inasmuch as the latter is an efficient vector of tularemia, it is not impossible that Otobius lagophilus, while not a direct vector, may play a part in nature as a reservoir of P. tularensis. Still other adult ticks from this experiment were maintained to study the persistence of tularemia organisms.

From October 13, 1957, the day the infecting host rabbit died, until August 20, 1959, the last day on which ticks of this experiment were tested, tularemia organisms were found in all ticks tested, a period of 676 days. It was concluded from these data that, once infected, ticks of this species remain infected for life.

Ornithodoros hermsi

Three ticks of this species which had been previously fed on a guinea pig dying of tularemia, were fed to repletion on deer mice (naked strain) in an attempt to transmit P. tularensis. No transmissions resulted. Ticks subsequently died of excessive heat.

STUDIES ON THE ECOLOGY OF WILDLIFE

Food Habits of Rodents

Certain rodents were reported by Vest and Marchette (1958)[1] to be readily infected with tularemia after ingesting infective rodent flesh. In order to determine the likelihood of wildlife obtaining infection in this manner, studies of the seasonal food habits of wildlife were conducted through the late winter and early summer months of this report period. The results indicate a variation of diet during each season in the various biotic communities studied.

The results showed the diet of the ubiquitous deer mouse followed the same general pattern in each of the 3 natural biotic communities. The diet in each case consisted mainly of seeds, with a minor component of arthropods during later winter and early spring, but gradually reversed the proportions later in the spring as more arthropods became available, Table 12.

TABLE 12. Food of Deer Mice. Upper figure is volumetric percentage; lower figure (in parentheses) represents percent frequencies of occurrence.

Community	Stomachs examined	Period	Seeds	Leaf	Arthropods	Nematodes
Vegetated Dune	20	March	82 (95)	2 (5)	16 (40)	
	7	April	55 (82)		45 (71)	
	9	May	20 (22)		78 (88)	2 (22)
Juniper Brush	3	March	66 (66)		33 (33)	
	8	April	22 (68)		78 (100)	
		May	15 (20)		82 (90)	3 (20)
Shadscale-budsage	18	March	86 (100)		13 (67)	1 (5)
	10	April	13 (30)		87 (100)	

[1] Vest, E. D. and N. J. Marchette, 1958. Transmission of *Pasteurella tularensis* among desert rodents through infective carcasses. Science. 128:363-364.

The Ord kangaroo rat's diet showed a similar pattern of change, Table 13. During March (prior to the budding of plants) the volume of seeds decreased in the stomach contents and leafy material from plants became the major constituent of the diet. In a few instances there were arthropod remains found in the rat's stomach, but they are predominantly vegetarian.

TABLE 13. Food of Ord kangaroo rats: Upper figure represents volumetric percentage; lower figure (in parentheses) represents frequencies of occurrence.

Community	Stomachs examined	Period	Seeds	Leaf	Arthropods	Nematodes
Vegetated Dune	20	March	98 (100)	2 (15)		
	36	April	82 (81)	13 (20)	2 (5)	3 (8)
	20	May	21 (55)	73 (95)	6 (30)	
Juniper Brush	10	March	81 (90)	19 (30)		
	8	April	30 (50)	64 (75)	6 (37)	
	14	May	22 (42)	65 (92)	12 (42)	1 (7)
Shadscale-budsage	10	March	93 (100)	2 (20)	5 (10)	
	9	April	83 (100)	3 (22)	14 (66)	

The chisel-toothed kangaroo rat's diet consisted primarily of leafy material, Table 14. During March there was a paucity of study material; however, they still consumed seeds, which would be necessary because the green plants were still dormant. Occasionally small quantities of arthropod remains were found in their stomach contents.

The food of the long-tailed pocket mouse during the latter part of April was about half seed, and half arthropod material, Table 14.

The harvest mouse diet included seeds, leaves, arthropods and nematodes during March. In April, the diet remained about the same except for the absence of leafy material, Table 14.

TABLE 14. Food of Other Rodents: Food of the chisel-toothed kangaroo rat, long-tailed pocket mouse, and harvest mouse in various biotic communities of Dugway Proving Ground. Upper figure is volumetric percentage; lower figure (in parentheses) represents frequencies of occurrence.

Community	Stomachs examined	Period	Seeds	Leaf	Arthropods	Nematodes
	Dipodomys microps - Chisel-toothed kangaroo rat					
Juniper Brush	3	April	100 (100)			
Mixed Brush	8	April	11 (12)	88 (87)	2 (12)	
Shadscale-budsage	8	March	36 (75)	63 (87)		
	10	April	16 (50)	81 (90)	3 (40)	
	Perognathus formosus - Long-tailed pocket mouse					
Mixed brush	13	April	47 (85)		53 (77)	
	Reithrodontomys megalotis - Harvest mouse					
Vegetated Dunes	11	March	48 (55)	26 (27)	9 (45)	17 (27)
	9	April	58 (78)		36 (67)	6 (11)

Chemical Control of Rodent Ectoparasites

Studies to determine the residual effect of dieldrin, a chlorinated hydrocarbon insecticide, sprayed at the rate of 0.75 pounds per acre, in a vegetated dune area with ground spray equipment, were continued during this report period. The Ord kangaroo rats, Dipodomys ordii, captured 907 days after application of the spray showed the residual effect of the dieldrin still produced a significant reduction of their ectoparasites. Nine hundred and seven days after the spraying, the total ectoparasite index of kangaroo rats from the sprayed plot was 15.12, while the index from the non-sprayed plot was 89.60. Table 15 shows the incidence of each group of ectoparasites 907 days after application. Fig. 4 shows the residual effect of the dieldrin on Ord kangaroo rat ectoparasites taken periodically from 340 to 907 days after application of spray.

TABLE 15. Control of Ectoparasites. Effects of dieldrin (0.75 pounds per acre) on rodent ectoparasites, 907 days after application.

Area	Ectoparasite Index				
	Fleas	Ticks	Mites	Lice	Total
Sprayed	0.02	7.4	1.0	6.7	15.12
Unsprayed	2.8	64.5	2.7	19.6	89.60

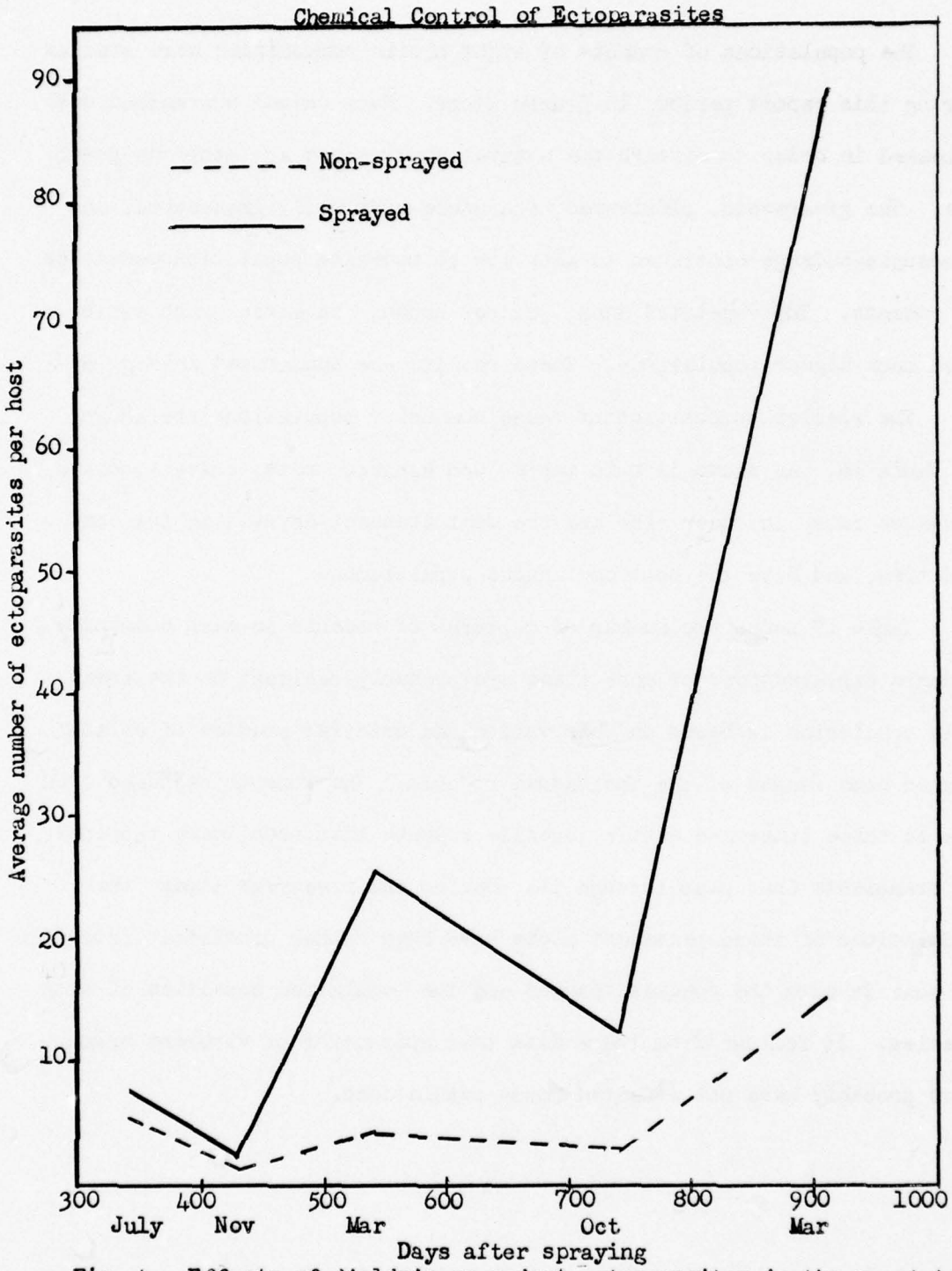

Fig. 4. Effects of dieldrin on rodent ectoparasites in the vegetated dune community (applied at the rate of 0.75 lbs. per acre with ground spray equipment).

Rodent Populations of Biotic Communities

The populations of rodents of eight biotic communities were studied during this report period, in 5-acre plots. Each animal was marked and released in order to disturb the natural populations as little as possible. The greasewood, pickleweed, shadscale-gray molly-greasewood, and shadscale-budsage continued to show low to moderate population densities of rodents. The vegetated dune, juniper brush, and mixed brush exhibited much higher populations. These results are summarized in Fig. 5.

The species composition of these community populations are shown in Table 16. As shown in this table, Ord kangaroo rats, chisel-toothed kangaroo rats, and deer mice are the most abundant animals in the communities, and have the most continuous populations.

Table 17 shows the number of captures of rodents in each community. Rodents captured four or more times are probably resident to the area. This conclusion is based on observation and detailed studies of established home ranges of the individual rodents. The rodents captured from one to three times are either juvenile rodents that soon leave the area, or transients that pass through it. During the five-year study, the populations of these permanent plots have been rather consistent from year to year in both the species trapped and the population densities of each species. It follows from these data that epizootics of virulent organisms probably have not affected these populations.

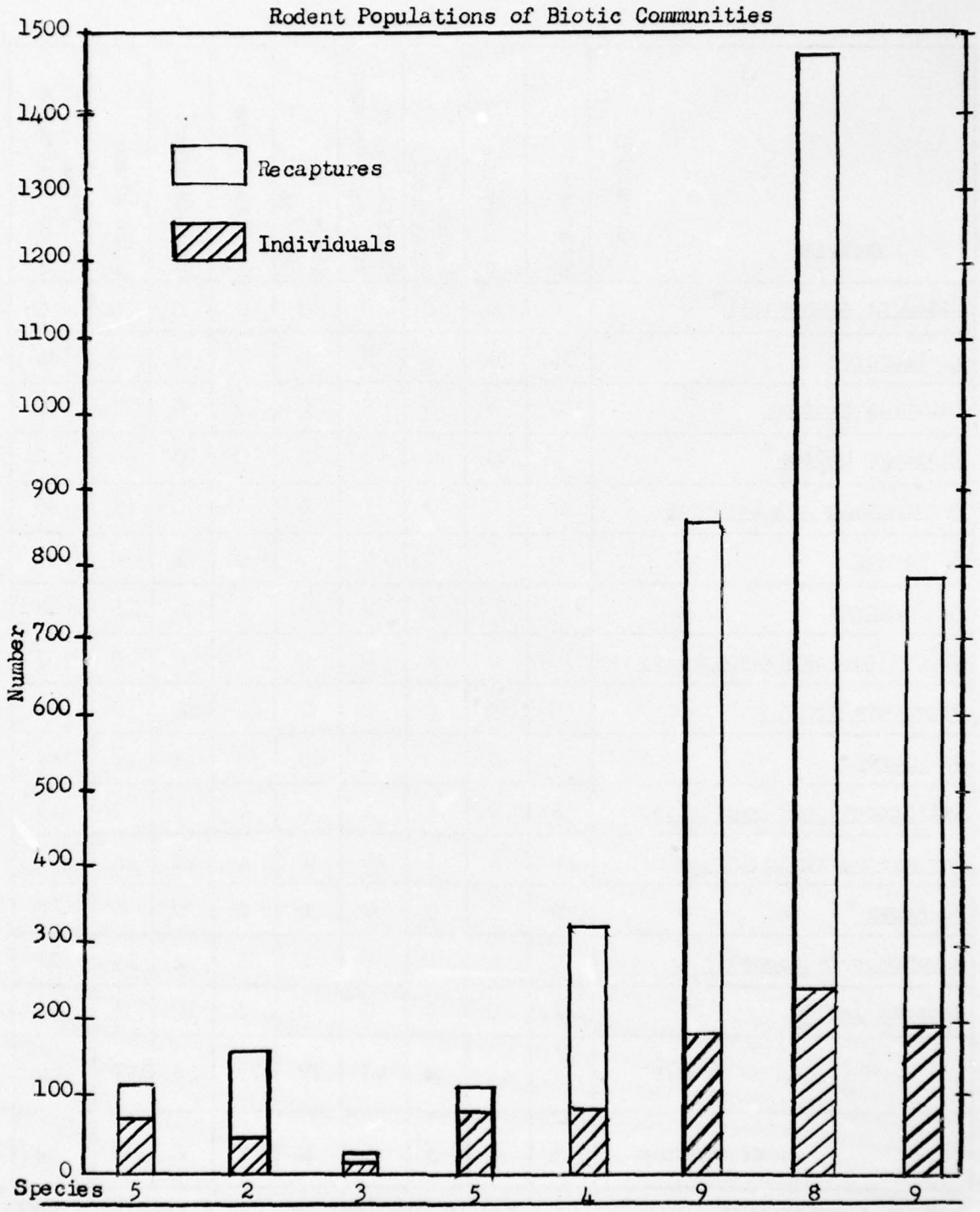

Fig. 5. Bar graph showing the number of individual rodents captured and the number of recaptures on 5-acre plots of 8 biotic communities during 1959.

TABLE 16. Rodent Composition of Biotic Communities Species composition of rodent populations of biotic communities during 1959.

ANIMALS / COMMUNITY	Greasewood	Pickleweed	Shadscale-gray molly	Shadscale-gray molly-greasewood	Shadscale-budsage	Vegetated Dune	Juniper brush	Mixed brush	Total Animal Captures
Citellus townsendii*	0	0	0	0	0	0	0	0	0
C. leucurus	35	0	0	0	9	7	33	0	84
Eutamias minimus	0	0	0	8	0	10	0	0	18
Thomomys bottae*	0	0	0	0	0	0	0	0	0
Perognathus longimembris	22	0	0	1	0	46	0	17	86
P. parvus	0	0	0	0	0	0	1	8	9
P. formosus	0	0	0	0	0	0	0	18	18
Microdipodops megacephalus	0	0	2	0	0	7	0	0	9
Dipodomys ordii	8	35	0	0	0	42	152	49	286
D. microps	0	0	0	9	68	50	3	69	199
Reithrodontomys megalotis	0	0	1	4	0	6	5	3	19
Peromyscus maniculatus	6	6	5	20	1	4	48	14	104
P. truei*	0	0	0	0	0	0	0	0	0
Onychomys leucogaster	0	0	0	0	1	5	2	13	21
Neotoma lepida	1	0	0	0	0	0	14	1	16
Totals	72	41	8	42	79	177	258	192	869
No. of Species	5	2	3	5	4	9	8	9	12

* These species were found during previous studies, but not during this year.

TABLE 17. Rodent Captures in Biotic Communities. Showing the captures of rodents in the biotic communities during the summer of 1959.

1959	No. of rodents captured				
Vegetation	Once	2 or 3 times	4 or more times*	Total individuals	Total Captures
Greasewood	38	28	5	72	128
Pickleweed	10	13	18	41	166
Shadscale-gray molly	4	2	2	8	19
Shadscale-gray molly-greasewood	20	12	10	42	108
Vegetated dune	57	39	81	177	858
Juniper brush	82	53	123	258	1488
Mixed brush	69	51	72	192	792
Shadscale-budsage	24	24	31	79	312
Total	304	222	342	869	3,871

* Rodents captured 4 or more times are considered resident to the area.

Community Distribution of Wildlife Collected for Disease Studies

The relationship of wildlife species and their habitat is shown in Table 18. The mixed brush community exhibits the greatest number of species and total individuals captured, while the shadscale-gray molly exhibits the least.

The locations of trapping stations in the natural habitat and in domestic areas are shown in Tables 19 and 20.

TABLE 18. Wildlife Disease Survey. Wildlife species collected for disease survey studies, tabulated according to the general habitat from which collected.

SPECIES	Buildings	Vegetated dunes	Mixed brush	Juniper Mountain	Juniper brush	Greasewood	Shadscale-budsage	Shadscale-gray molly	Shadscale-gray molly-greasewood	Marsh	TOTAL
MAMMALS											
Lepus californicus		30	46	8	47	210	1		66		408
Sylvilagus nuttallii			2		1	1			1		5
S. audubonii			7		3	12			3		25
Citellus leucurus	3	63	74	4	5	21	11		31		212
Eutamias minimus		10	21	4					8		43
E. dorsalis				23							23
Perognathus longimembris		24	42			7	4		6		83
P. parvus			43	45		2					90
P. formosus		1	41	16			2		1		61
Microdipodops megacephalus		5									5
Dipodomys ordii		130	53	29	17	16	2		20		267
D. microps		52	77	16		60	16		24		245
Reithrodontomys megalotis	1	8	15	6		10		4		61	105
Peromyscus crinitus	2		41	38							81
P. maniculatus	115	15	180	161	5	49	4	3	10	2	544
P. truei	4			134							138
Onychomys leucogaster	2	2	2				1		1		8
Neotoma lepida	3		22	17	2	1					46
N. cinerea				1							1
Ondatra zibethicus										1	1
Microtus montanus										4	4
Mus musculus	1									1	2
Erethizon dorsatum					2						2
Canis latrans		1	1						1		3
Taxidea taxus		1	1		1				1		4
Lynx rufus		2	1								3
Felis catus	6										6
Odocoileus hemionus				13							13
BIRDS											
Buteo jamaicensis				2							2
Aquila chrysaetos								2			2
Columba livia								2	1		3
Corvus corax					2			2	2		6
Total Species	9	14	18	16	10	10	8	5	16	5	32
Total Number	137	144	669	517	85	389	41	13	177	69	2,441

TABLE 19. Vertebrates Collected. Number of lagomorphs, rodents and vertebrates collected from each of the major wildlife collecting areas on Dugway Proving Ground and adjacent areas

Collecting Area	Dugway Proving Ground			
	Lagomorphs	Rodents	Other vertebrates	Total
Camelback Mountain	1	81	7	89
CD 22		15		15
Dugway Valley	1	54	3	58
Government Creek	10	87	9	106
Granite Mountain	1	42		43
Little Davis Mountain	35	62	5	102
Old River Bed	43	29		72
South Cedar Mountain	4	45	4	53
Test Grid		25		25
Wig Mountain	37	30	1	68
Adjacent Areas				
Callao	59	329	7	395
Cedar City		67		67
Clover	1	73		74
Deep Creek	16			16
Duchesne		39		39
Dugway Mountain	3	19		22
Fillmore	3	69		72
Fish Springs	1	52	1	54
Gandy	18			18
Gold Hill	42	129		171
Hanksville		4		4
Johnson Pass	4	29	5	38
Lookout Pass		46	2	48
North Skull Valley	35	93		128
North Wendover	44	173		217
North Wig Mountain		30		30
Old River Bed	18			18
Simpson Mountain	3		1	4
South Skull Valley	14	20		34
South Wendover	6	106		112
South Willow	1	29		30
Trout Creek	24			24
Utah Lake		13		13
Vernon	14	37		51
Total	438	1,827	45	2,310

TABLE 20. Rodents Collected in Dugway Buildings. The following rodents were captured in and adjacent to the following inhabited buildings on Dugway Proving Ground.

Locality / Rodents	*Citellus leucurus* Antelope ground squirrel	*Reithrodontomys megalotis* Harvest mouse	*Peromyscus crinitus* Canyon mouse	*P. maniculatus* Deer mouse	*P. truei* Pinyon mouse	*Onychomys leucogaster* Grasshopper mouse	*Neotoma lepida* Wood rat	*Mus musculus* House mouse	TOTAL
BAKER AREA									
Bldg 2028				2				1	3
DOG AREA									
Bldg 4010				11					11
4090				2					2
EASY AREA									
Animal clinic				4					4
Headquarters				1					1
GPI-1 AREA									
Bldg 3200				13					13
3204	3	1		13	4	2	3		26
3206			2	32					34
3208				15					15
3210				7					7
Flea Cellar				6					6
Garbage Pit				5					5
Rabbit house				2					2
Total	3	1	2	115	4	2	3	1	131

Native Mammals Collected Alive for Experimental Use

During this year, 1,007 native mammals have been collected for experimental use, food habit studies, or for colonization. There were one species of rabbit, 17 species of rodents, and 2 species of carnivores, as listed in Table 21.

TABLE 21. Live Mammals Collected

Lagomorphs	
Sylvilagus audubonii - Audubon cottontail	42
Rodents	
Citellus leucurus - Antelope ground squirrel	40
Eutamias minimus - Least chipmunk	8
E. dorsalis - Cliff chipmunk	46
Perognathus longimembris - Little pocket mouse	42
P. parvus - Great Basin pocket mouse	177
P. formosus - Long-tailed pocket mouse	27
Microdipodops megacephalus - Kangaroo mouse	2
Dipodomys ordii - Ord kangaroo rat	324
D. microps - Chisel-toothed kangaroo rat	96
Reithrodontomys megalotis - Harvest mouse	58
Peromyscus crinitus - Canyon mouse	2
P. maniculatus - Deer mouse	71
P. truei - Pinyon mouse	19
Onychomys leucogaster - Grasshopper mouse	17
Neotoma lepida - Desert wood rat	26
N. cinerea - Bushy-tailed wood rat	1
Microtus montanus - Meadow mouse	9
Total	1,007

Population Fluctuations of the Jack Rabbit

Studies on the population fluctuations of the jack rabbit, Lepus californicus deserticola, were continued in the selected areas near Camelback Mountain, on Dugway Proving Ground. There were more jack rabbits observed during the monthly censuses this year than in any other year that has been studied since 1953.

COLONIZATION OF NATIVE ECTOPARASITES

Rearing Colonies

Six native species of ticks and 5 native species of fleas have been successfully reared and maintained throughout the period of this report. One of the tick species, Haemaphysalis leporis-palustris, was completely lost when the laboratory air conditioner failed during the summer. One of the flea colonies dwindled in numbers, but was restocked by new specimens taken from natural hosts.

A flea species, Meringis parkeri, was successfully reared in nests with kangaroo rats for the first time in this laboratory, but development of the colony was slow.

During the summer of 1958, samples of nesting litter from squirrel flea rearing units were placed in earthenware containers and stored in an underground rearing room, 34°-69°F. It was suspected that the summer squirrel flea, Hoplopsyllus anomalus, must winter over in one of its immature stages. In February a portion of debris from one of these units was brought into the laboratory and heated to 70°-80°F. In two days, larvae appeared in the litter, and on the third day about 6 adults appeared. Apparently the cooler conditions in the underground room served to retard development, aiding the fleas to survive the winter in the immature stages. It is interesting to note that both hatching and metamorphosis in this species were induced by these lower temperatures during the winter time when this flea is not normally active.

It seemed probable that temperature might help to regulate the seasonal development of other fleas, also. Because Rhadinopsylla heiseri has been found on antelope ground squirrels during winter months only, an attempt

was made to study the effect of temperature on its development. Litter and debris from squirrel nests infested by this flea were taken during the winter, and stored. The nests were kept in both underground and laboratory rearing units from one winter to the next. No larvae nor adults could be found in the nest litter, either in summer or the following winter. Apparently suitable rearing conditions had not been provided. In addition, fleas were implanted on squirrels in artificial burrow cases in the laboratory. Fleas were also placed with squirrels in nest boxes, in the underground room. From these nest boxes, which were covered with earth, squirrels were free to leave their nests at will and, by means of the enclosed tunnel, reach an outdoor cage to bask during warm periods of the day. Under these conditions, fleas survived for a maximum of 80 days, and larvae were found in the litter.

Foxella ignota placed with a pocket gopher in a mouse cage partially filled with prepared sandy litter containing larval nutrient media, survived a maximum of 45 days, but larvae were not found in the cage litter during nor after the above period.

In life history studies with ticks, feeding times were established on approximately 250 O. hermsi larvae. Molting time was recorded at 6-17 days after feeding. In the laboratory, oviposition by O. parkeri occurred during April.

ARTIFICIAL FEEDING OF FLEAS

An experiment was designed to develop techniques of feeding fleas of the species Orchopeas leucopus through artificial membranes. Citrated rabbit blood was pooled in a plastic receptacle and warmed in a water

bath held at 34.7° to 35.5°C. A skin membrane from a pinyon mouse was stretched over the end of a flea-holding chamber which was lowered to rest over the pooled citrated blood.

Of 39 adult laboratory reared fleas never exposed to a host, 28 (72%) fed. The above results have since been duplicated in further experiments using the same device.

EFFECTS OF HUMIDITY ON FLEA COLONIZATION

An experiment was designed and conducted to assess the effects of humidity on the development of O. leucopus from egg to adult stage. In this study a wooden chamber 16x8x9 inches, with a hinged lid comprising one half of the entire top area of the chamber, was used. At the enclosed end of the chamber, four small metal water containers served as a source of moisture. Four jars each containing 250 flea larvae in rearing medium were situated within the chamber as follows: (a) placed adjacent to water containers; (b) 4 inches from jar (a); (c) 4 inches from (b); and (d) adjacent to outside opening of box and partially exposed to outside drying effects of room air. A cloth mesh was placed over each jar to prevent escape of adult fleas.

Counts were made after 21 days, as follows: Jar (a) contained 157 adults; jar (b) 11; jar (c) 7; and jar (d) none. Since Jar (a), having the highest number of adults at the end of the time period, had only approximately 2/3 of the total number of fleas initially introduced into the experiment, it is possible that all of the fleas were not counted. However, the results show a relationship between development of immature stages of fleas and the moisture content of the atmosphere around them.

EPIZOOLOGICAL SURVEY OF WILDLIFE AND LIVESTOCK

During the current report period a systematic collection of wild animals was made to study the incidence of enzootic diseases in native animals and livestock. The areas sampled extended from eastern Nevada to Stockton, Utah; north to Lucin, Utah; and south to Gandy and Vernon, Utah. From each sampling area the animals were pooled according to species and community type. The tissues of the wildlife specimens were examined for plague, P. pestis; tularemia, P. tularensis; anthrax, Bacillus anthracis; brucellosis, Brucella sp.; Q fever, C. burneti; and Rocky Mountain spotted fever, Rickettsia rickettsii. The serum specimens of wildlife and domestic animals were tested individually for Rocky Mountain spotted fever and Q fever complement fixing antibodies, and for tularemia and brucella agglutinins.

The techniques used for isolation of the infectious organisms and the serological methods and interpretations of results corresponded to those of the Rocky Mountain Laboratory. The annual reports of Dr. H. G. Stoenner to the Chief, E and E Branch, BW Operations Division, Dugway Proving Ground, Dugway, Utah, dated 17 May, 1955 to 20 July, 1956, summarize these methods. One modification of previous methods was the use of commercial antigens, since the antigens used by the prior contractor were not available in the quantities needed. The Q-fever, American (Nine Mile) strain, and Rocky Mountain spotted fever antigens were obtained from Lederle Laboratories. The Br. abortus tube agglutination antigen was provided by the U. S. Department of Agriculture. Antigen for the P. tularensis tube agglutination test was prepared from strain Schu A according to the procedures of Dr. D. B. Lackman, Rocky Mountain Laboratory.

On the basis of a positive antibody response in guinea pigs inoculated with tissue homogenates of collected animals, the apparent presence of the organisms studied is recorded in Table 24. Their distribution in the collection areas is summarized in Table 25. Similar data relating to the apparent presence of the causative agents of Rocky Mountain spotted fever, Q fever, and tularemia, in pools of ectoparasites are presented in Table 27.

Q Fever, Coxiella burneti:

The increase in incidence and distribution of Q fever since 1954 was summarized by Vest, et al., 1959.[1] The diagnostic results obtained during the present report period tend to indicate a greater incidence of Q fever and extend the known infected rodent species to include the pinyon mouse, P. truei. Of 3,309 wildlife serum samples tested, 233 (7%) had complement fixing antibody titers for Q fever of 1:16 or greater, Tables 22 and 23.

In the apparent endemic areas of Fish Springs, Gold Hill, North Wendover and South Wendover, the incidence of Q fever complement fixation antibody titers of 1:16 or greater in wildlife sera has increased as much as six-fold over the 1958 results.

Evidence of the presence of C. burneti in 15 wildlife tissue pools was demonstrated by Q fever complement fixing antibodies of 1:16 or greater in guinea pigs after inoculation of tissue homogenates, Tables 24 and 25. Rickettsiae were isolated from five of the original tissue pools by reinoculation into guinea pigs or hamsters, as indicated in Table 26. Two of these strains, 2145 and 2269, have been cultured in the yolk sacs of 7-day old

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[1] Vest, E. D., F. T. Gardner, B. D. Thorpe, R. W. Sidwell, and R. Ushijima. Epizoological survey of certain endemic diseases in the southern part of the Great Salt Lake Desert. Ecology and Epizoology series, No. 42, June 30, 1959. Ecological Research, University of Utah.

embryonated hen's eggs. Second passage egg yolk sacs were harvested, homogenized, and injected intraperitoneally into 5 guinea pigs. Of the 5 guinea pigs challenged with strain 2145, none developed a temperature of 104°F or greater during the first two weeks following the challenge. One of the 5 guinea pigs challenged with strain 2269 developed a fever of 104.2°F on the 8th day following challenge; another animal exhibited a temperature of 104.3°F on the 12th day following challenge. These febrile responses persisted for less than 24 hours. The remaining 3 challenged guinea pigs exhibited no temperatures of 104°F or greater. Other overt symptoms of infection were not observed. The complement fixing antibody titers of these challenged guinea pigs are summarized in Figs. 6 and 7. Characteristics of these C. burneti isolated did not appear to be significantly different from those isolated from animals collected in the same areas, as reported by Stoenner, et al., (1959),[1] and Stoenner and Lackman (1960).[2]

The apparent presence of C. burneti was noted in a pool of fleas from animals collected in North Skull Valley and a pool of fleas from animals collected in South Cedar Mountains, Table 27. This was determined on the basis of development of complement fixing antibody in the serum from guinea pigs with these flea pool homogenates.

All of the 196 sheep sera tested for Q fever complement fixing antibodies were negative, while 91(4.6%) of the 1,965 cattle sera tested were found to have Q fever complement fixing antibody titers of 1:16 or greater, Table 28.

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[1] Stoenner, H. G., R. Holdenried, D. Lackman, and J. S. Orsborn, Jr., 1959. The occurrence of Coxiella burnetii, Brucella and other pathogens among fauna of the Great Salt Lake Desert of Utah. Amer. J. Trop. Med. Hyg. 8(5):590-596.

[2] Stoenner, H. G. and D. B. Lackman. 1960. The biologic properties of Coxiella burnetii isolated from rodents collected in Utah. Amer. J. Hyg. 71(1): 45-51.

TABLE 22. Incidence of Disease as Indicated by Serology. Incidence of Coxiella burneti (Q fever), Rickettsia rickettsii (Rocky Mountain spotted fever), and Pasteurella tularensis (tularemia), infections as determined by antibody titers in the sera of native animal species in Western Utah and Eastern Nevada.

Species		Q Fever		RMSF		Tularemia	
	Number examined	Number positive	Per cent	Number positive	Per cent	Number positive	Per cent
Lepus californicus							
Black-tailed jack rabbit	510	57	11.1	269	52.7	1	0.02
Sylvilagus nuttallii							
Nuttall cottontail	4	1	25.0	2	50.0	-	-
S. audubonii							
Audubon cottontail	26	5	19.0	4	15.0	-	-
Citellus leucurus							
Antelope ground squirrel	236	7	3.0	79	33.0	-	-
Eutamias minimus							
Least chipmunk	53	-	-	3	5.6	1	1.8
E. dorsalis							
Cliff chipmunk	27	1	3.7	7	25.9	-	-
Perognathus longimembris							
Little pocket mouse	68	1	1.4	5	7.3	-	-
P. parvus							
Great Basin pocket mouse	97	15	15.4	14	14.4	-	-
P. formosus							
Long-tailed pocket mouse	65	1	1.5	9	13.8	-	-
Microdipodops megacephalus							
Kangaroo mouse	10	-	-	1	10.0	-	-
Dipodomys ordii							
Ord kangaroo rat	416	30	7.2	83	19.9	-	-
D. microps							
Chisel-tooth kangaroo rat	406	22	5.4	88	21.6	-	-
Reithrodontomys megalotis							
Harvest mouse	102	9	8.8	9	8.8	-	-
Peromyscus crinitus							
Canyon mouse	75	3	4.0	15	20.0	-	-
P. maniculatus							
Deer mouse	957	63	6.5	176	18.3	3	0.7
P. truei							
Pinyon mouse	139	4	2.8	28	14.7	3	2.1
Onychomys leucogaster							
Grasshopper mouse	8	1	12.5	-	-	-	-
Neotoma lepida							
Wood rat	53	5	9.4	16	20.1	-	-
Ondatra zibethicus							
Muskrat	1	-	-	-	-	-	-
Microtus montanus							
Meadow mouse	4	-	-	-	-	-	-
Sub-total	3,257	225		808		8	

TABLE 22. Incidence of Disease as Indicated by Serology (continued)

Species	Number examined	Q Fever		RMSF		Tularemia	
		Number positive	Per cent	Number positive	Per cent	Number positive	Per cent
Sub-total	3,257	225		808		8	
Mus musculus House mouse	1	-	-	-	-	-	-
Erethizon dorsatum Porcupine	1	-	-	-	-	-	-
Canis latrans Coyote	3	1	33.3	1	33.3	-	-
Vulpes macrotis Kit fox	1	-	-	-	-	-	-
Taxidea taxus Badger	4	-	-	-	-	1	25.0
Spilogale gracilis Spotted skunk	1	-	-	-	-	-	-
Lynx rufus Bob cat	3	-	-	-	-	-	-
Felis catus Domestic cat	6	-	-	-	-	-	-
Odocoileus hemionus Mule deer	15	7	46.6	-	-	2	13.3
Buteo jamaicensis Red-tailed hawk	2	-	-	-	-	-	-
Aquila chrysaetos Golden eagle	2	-	-	-	-	-	-
Columba livia Domestic pigeon	3	-	-	-	-	-	-
Corvus corax Raven	7	-	-	-	-	-	-
Euphagus cyanocephalus Brewer blackbird	1	-	-	-	-	-	-
Zonotrichia l. gambelii Gambel sparrow	2	-	-	-	-	-	-
Total	3,309	233	7.0	809	24.4	11	0.4

TABLE 23. Distribution of Disease as Indicated by Serology. Incidence of Coxiella burneti (Q Fever), Rickettsia rickettsii (Rocky Mountain spotted fever), and Pasteurella tularensis (tularemia) infections as determined by antibody titers in native animal sera from the 33 major areas trapped in Utah and Nevada.

Area	Number examined	Q Fever		RMSF		Tularemia	
		Positive	Per cent	Positive	Per cent	Positive	Per cent
Callao	367	33	9.8	86	23.4	1	0.3
Camelback Mountain	93	2	2.1	1	1.0	-	-
CD 22	20	1	5.0	3	15.0	-	-
Cedar City *	65	3	4.6	18	27.6	-	-
Clover	144	11	7.6	24	16.6	-	-
Deep Creek	1	1	100.0	1	100.0	-	-
Duchesne	38	-	-	1	2.6	-	-
Dugway Mountain	18	-	-	5	27.7	-	-
Dugway Valley	51	2	3.9	9	17.6	-	-
Fillmore *	70	5	7.1	11	15.7	-	-
Fish Springs	43	11	25.5	18	41.8	-	-
Gandy *	18	-	-	4	22.2	-	-
Gold Hill	164	22	13.4	95	57.9	-	-
Government Creek	454	18	4.0	96	21.1	1	0.2
Granite Mountain	35	-	-	8	22.8	-	-
Hanksville *	4	-	-	-	-	-	-
Johnson Pass	37	4	10.8	5	13.5	3	8.1
Little Davis Mountain	122	9	7.3	31	25.4	1	1.6
Lookout Pass	45	4	8.8	13	28.8	4	8.8
North Skull Valley	240	12	5.0	78	32.5	-	-
North Wendover	196	35	17.8	80	40.8	-	-
Old River Bed	288	7	2.4	58	20.1	-	-
Sheeprock Mountain	1	-	-	-	-	-	-
Simpson Mountain	18	1	5.5	3	16.6	-	-
South Cedar Mountain	279	8	2.8	44	15.7	-	-
South Skull Valley	47	3	6.3	12	25.5	-	-
South Wendover	104	17	16.3	24	23.0	-	-
South Willow *	30	3	10.0	13	43.3	-	-
Test Grid	36	1	2.7	2	5.5	-	-
Trout Creek	24	1	4.1	7	29.1	-	-
Vernon	106	1	0.9	7	6.6	1	1.0
Wig Mountain	127	14	13.2	52	40.9	-	-
Wildcat Mountain	24	1	4.1	-	-	-	-
Total	3,309	233	7.0	809	24.4	11	0.4

* Outside regular sampling areas.

TABLE 24. Incidence of Disease Organisms in Wildlife. Incidence of Coxiella burneti (Q Fever), Rickettsia rickettsii (Rocky Mountain spotted fever), Pasteurella tularensis (tularemia)*, and Brucella sp. (brucellosis), in 22 species of native animals, as indicated by antibody titers in guinea pigs 42 days after parenteral injection of animal tissues.

Species	Number of animals examined	Q Fever		RMSF	
		Number of Positive pools	Per cent	Number of Positive pools	Per cent
Lepus californicus					
Black-tailed jack rabbit	442	2	0.5	65	14.7
Citellus leucurus					
Antelope ground squirrel	221	2	0.9	15	6.7
Eutamias minimus					
Least chipmunk	47	-	-	3	6.3
E. dorsalis					
Cliff chipmunk	20	-	-	2	10.0
Perognathus longimembris					
Little pocket mouse	85	2	2.3	6	7.1
P. parvus					
Great Basin pocket mouse	89	-	-	-	-
P. formosus					
Long-tailed pocket mouse	62	-	-	-	-
Microdipodops megacephalus					
Kangaroo mouse	5	-	-	2	3.2
Dipodomys ordii					
Ord kangaroo rat	250	1	0.4	24	10.0
D, microps					
Chisel-tooth kangaroo rat	249	4	1.6	18	7.2
Reithrodontomys megalotis					
Harvest mouse	106	-	-	2	1.8
Peromyscus crinitus					
Canyon mouse	80	-	-	4	5.0
P. maniculatus					
Deer mouse	611	-	-	35	5.7
P. truei					
Pinyon mouse	134	1	0.7	5	3.7
Onychomys leucogaster					
Grasshopper mouse	8	-	-	-	-
Neotoma lepida					
Wood rat	46 **	-	-	3	6.5
N. cinerea					
Bushy-tailed wood rat	1	-	-	-	-
Microtus montanus					
Meadow mouse	4	-	-	-	-
Erethizon dorsatum					
Porcupine	3	-	-	2	66.6
Canis latrans					
Coyote	2	1	50.0	2	100.0
Felis catus					
Domestic cat	6	-	-	1	16.6
Odocoileus hemionus					
Mule deer	10	-	-	2	20.0
Total	2,481	15	0.5	193	7.7

* There were no isolations of P. tularensis, nor any serological evidence of tularemia in the tissue injected guinea pigs.

** Brucella neotomae (strain 9F201) was isolated from the South Wendover area by direct plating of the tissues on agar. No detectable infection was manifest in the tissue injected guinea pigs.

TABLE 25. Distribution of Disease Organisms. Incidence of Coxiella burneti (Q Fever), Rickettsia rickettsii, (Rocky Mountain spotted fever), Pasteurella tularensis (tularemia)*, and Brucella sp. (brucellosis), organisms, by locality, in native animals collected from 31 areas in Western Utah and Eastern Nevada, as determined by antibody titers in guinea pigs 42 days after parenteral injection of the animal tissues.

Area	Number of animals examined	Q Fever		RMSF	
		Number of positive pools	Per cent	Number of positive pools	Per cent
Callao	371	3	0.8	49	13.2
Camelback Mountain	84	-	-	4	4.7
CD 22	20	-	-	-	-
Cedar City	64	-	-	5	7.8
Clover	72	-	-	-	-
Duchesne	30	-	-	1	3.3
Dugway Mountain	23	-	-	-	-
Dugway Valley	72	2	2.7	13	18.0
Fillmore	72	-	-	1	1.3
Fish Springs	50	1	2.0	2	4.0
Gandy	14	-	-	1	7.1
Gold Hill	179	4	2.2	27	15.0
Government Creek	292	-	-	17	5.8
Granite Mountain	38	-	-	1	2.6
Hanksville	4	-	-	-	-
Johnson Pass	35	-	-	2	5.7
Little Davis Mountain	122	-	-	4	3.2
Lookout Pass	47	-	-	-	-
North Skull Valley	132	-	-	1	0.7
North Wendover	212	1	0.4	15	7.1
Old River Bed	104	-	-	13	12.5
Simpson Mountain	3	-	-	1	33.3
South Cedar Mountain	49	-	-	1	2.0
South Skull Valley	29	-	-	5	17.2
South Wendover	117 **	-	-	6	5.1
South Willow	29	-	-	1	3.4
Test Grid	20	-	-	3	15.0
Trout Creek	20	1	5.0	2	10.0
Utah Lake	13	-	-	-	-
Vernon	51	-	-	5	9.8
Wig Mountain	113	-	-	13	11.5
Total	2,481	15	0.5	193	7.7

* There were no indications of P. tularensis, nor any serological evidence of tularemia in the tissue-injected guinea pigs.

** Brucella neotomae (strain 9F 201) was isolated from a wood rat, Neotoma lepida, by direct plating of the tissues on agar. No detectable infection was manifest in the tissue-injected guinea pigs.

TABLE 26. *Coxiella burneti* Isolations

Q Fever strain	Rodent species	Area of capture	Confirmation animal	Maximum febrile response	Maximum CF Titer	Spleen impression smear
1517	*Perognathus longimembris*	Callao	Hamster	none	1/64	+
1471	*Dipodomys ordii*	Dugway Valley	Hamster	none	1/64	+
1619	*D. microps*	Gold Hill	Hamster	none	1/64	+
2145	*Lepus californicus*	Trout Creek	Guinea pig	none	1/128	+
2269	*D. microps*	Callao	Guinea pig	none	1/512	+

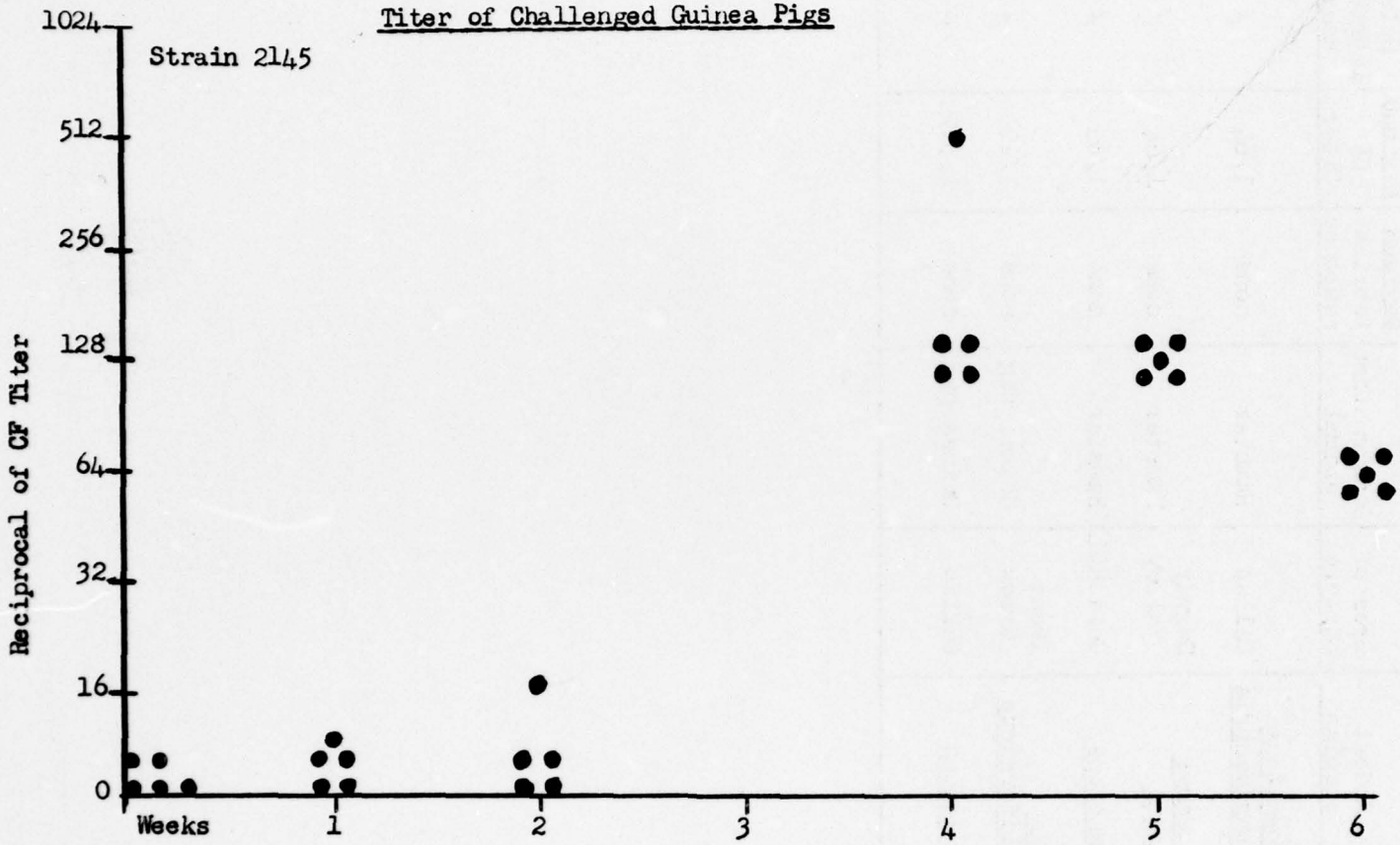

Fig. 6. CF antibody response of 5 guinea pigs challenged intraperitoneally with 1 ml of undiluted yolk sac homogenate of Q Fever strain 2145

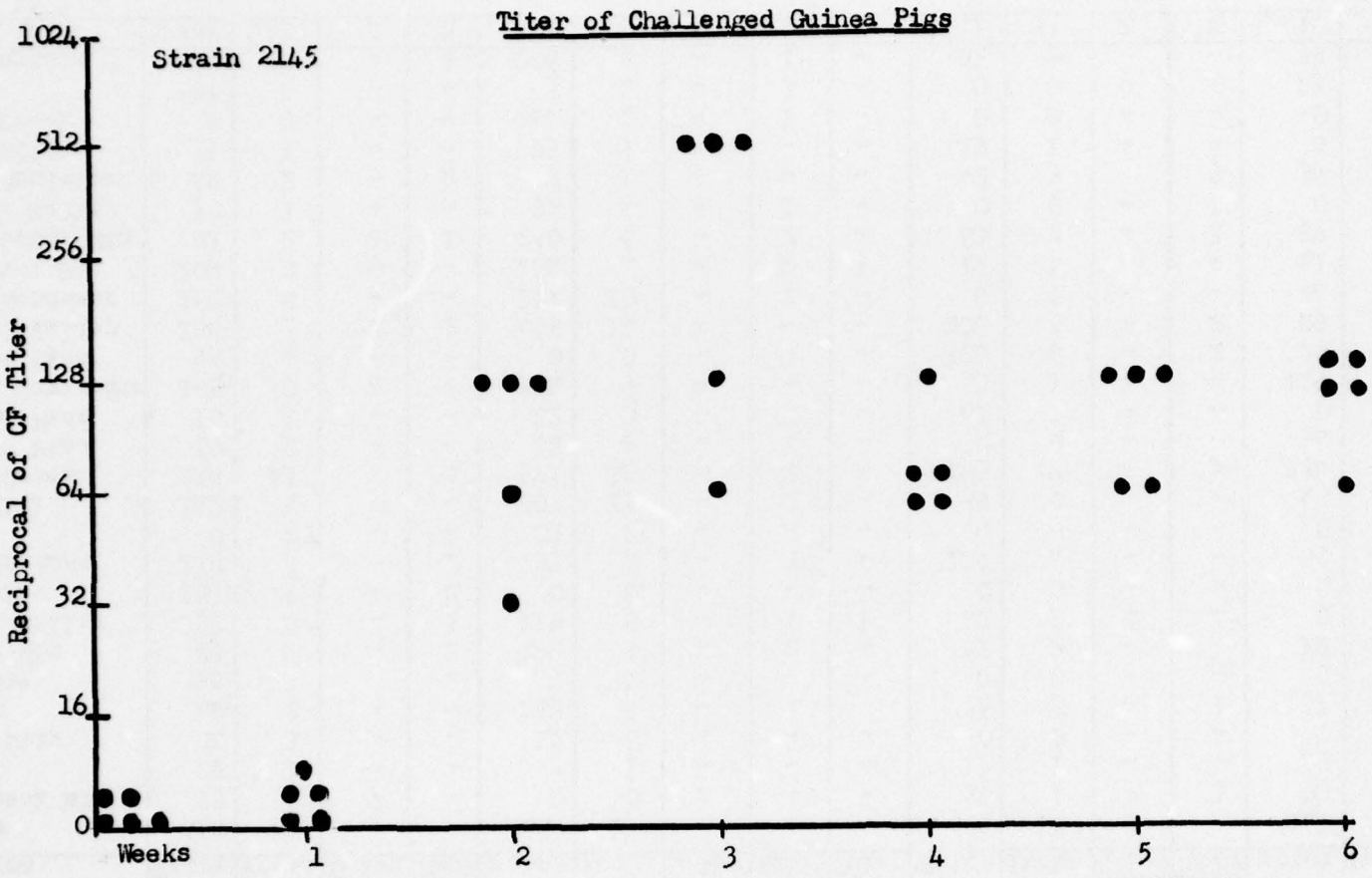

Fig. 7 CF antibody response of 5 guinea pigs challenged intraperitoneally with 1 ml of undiluted yolk sac homogenate of Q fever strain 2145.

TABLE 27. Disease Organisms in Native Ectoparasites. Incidence of Coxiella burneti (Q Fever), Rickettsia rickettsii (Rocky Mountain spotted fever), and Pasteurella tularensis (tularemia), organisms in ectoparasite pools from native animals collected in Western Utah and Eastern Nevada. Complement fixation titers of 1/16 or greater and tularemia agglutination titers of 1:40 or greater in injected guinea pigs were considered positive.

	Fleas				Ticks					Mites				Lice			
	No.	No.	Pos. pools		No.	No.	No. Pos. pools			No.	No.	Pos. pools		No.	No.	Pos. pools	
Area	Ectos	Pools	Q Fev	RMSF	Ectos	Pools	Q Fev	RMSF	Tular	Ectos	Pools	Q Fev	RMSF	Ectos	Pools	Q Fev	RMSF
Callao	550	17	-	-	608	17	-	1	-	509	10	-	-	141	5	-	2
Camelback Mtn	93	3	-	-	0	0	-	-	-	32	1	-	1	72	1	-	-
CD 22	27	1	-	-	3	1	-	-	-	11	1	-	-	49	1	-	-
Cedar City	84	3	-	2	12	6	-	-	-	0	0	-	-	0	0	-	-
Clover	44	2	-	-	253	6	-	1		76	3	-	1	31	3	-	-
Duchesne	95	3	-	2	3	1	-	-	-	0	0	-	-	0	0	-	-
Dugway Mtn	83	2	-	2	122	3	-	2	-	62	2	-	-	12	2	-	-
Dugway Valley	257	4	-	1	119	5	-	1	1	12	1	1	1	0	0	-	-
Fillmore	137	5	-	2	0	0	-	-	-	0	0	-	-	0	0	-	-
Fish Springs	218	5	-	2	197	5	-	3	-	129	4	-	-	63	2	-	-
Gandy	0	0	-	2	23	2	-	-	-	0	0	-	-	0	0	-	-
Gold Hill	147	4	-	-	807	15	-	2	-	39	2	-	-	42	2	-	1
Govt. Creek	418	13	-	4	571	14	-	2	-	195	7	-	3	336	5	-	2
Granite Mtn.	59	2	-	-	42	2	-	1	-	0	0	-	-	94	2	-	1
Johnson Pass	15	1	-	-	22	2	-	1	-	41	1	-	-	0	0	-	-
Little Davis Mtn	105	10	-	-	304	9	-	-	-	53	1	-	-	129	3	-	-
Lookout Pass	95	4	-	-	0	0	-	-	-	504	6	-	2	29	1	-	1
N Skull Valley	156	3	1	2	465	12	-	-	-	206	5	-	2	29	2	-	1
North Wendover	262	8	-	-	271	10	-	2	-	0	0	-	-	84	2	-	-
Old River Bed	109	2	-	-	104	4	-	2	1	17	1	-	-	41	1	-	-
South Cedar Mtn	81	2	1	1	256	6	-	2	-	63	2	-	1	57	2	-	-
S Skull Valley	38	1	-	-	92	4	-	1	-	0	0	-	1	0	0	-	-
South Wendover	67	2	-	2	7	1	-	-	-	92	3	-	3	36	2	-	-
South Willow	39	1	-	-	93	3	-	-	-	19	1	-	-	0	0	-	-
Trout Creek	0	0	-	-	44	2	-	1	-	0	0	-	-	0	0	-	-
Vernon	87	4	-	-	3	1	-	1	-	0	0	-	-	23	1	-	-
Wig Mountain	309	10	-	1	206	8	-	1	-	81	2	-	-	35	1	-	-
Total	3,585	112	2	23	4,626	134	0	24	2	2,141	53	1	12	1,410	38	0	9

Rocky Mountain Spotted Fever, Rickettsia rickettsii.

During the year 1959 there was an increase in the number of wildlife sera exhibiting complement fixing antibody reacting with Rocky Mountain spotted fever (RMsf) antigen, Tables 22 and 23. The increased incidence tends to be localized in the areas of Callao, Dugway Valley, Fish Springs, Gold Hill, Old River Bed, North Skull Valley, North Wendover, South Wendover, and Wig Mountain. In the remaining sampling areas, the incidence of RMsf appeared to be near that of previous years. However, too few animals were sampled and tested to draw significant conclusions. The incidence of these antibodies was greatest in the black-tailed jack rabbit, the ground squirrel, and the two species of kangaroo rats. A lesser increase in the number of individuals with serum antibody was noted in the case of deer mice collected from the above mentioned areas. There was also a noticeable increase in the incidence of positive serum samples from the canyon mouse, the pinyon mouse, and the desert wood rat. The incidence of RMsf positive sera from other species of wildlife showed no great divergence from past observations.

During the same period there has been a significant increase in the number of wildlife infected with RMsf as determined by antibody titers of 1:16 or greater in guinea pigs challenged with tissue homogenates, Tables 24 and 25. The number of wildlife animals which exhibited evidence of infection increased 15% above the previous year's sampling. This increased incidence in wildlife infections appeared to occur in the same areas as those where increased numbers of animals exhibiting antibodies were trapped.

It may be noted that the Callao, Dugway Valley, Fish Springs, Gold Hill, and North Skull Valley areas, according to previous annual reports, have had a greater number of animals infected than other sampled areas. It

appears that wildlife in the Callao, Dugway Valley and Fish Springs areas may have recently experienced an epizootic of this disease. It is probable that the wildlife from the Old River Bed, Wig Mountain, Granite Mountain, and Wendover areas may also have been involved.

The number of ectoparasite pools containing RMsf rickettsiae as determined in these studies apparently was not greater than found in previous years, Table 27.

Cattle sera examined during the report period showed an apparent increase in incidence of RMsf antibody titers of 1:16 or greater. This was in comparison to data collected on relatively few samples obtained in previous years. Eighty-three of 1,964 cattle sera and five of 196 sheep sera tested this year were found to be positive, Table 28. No positive sera were found in 176 cattle specimens tested the previous year.

TABLE 28. Serological Evidence of Disease in Livestock. The incidence of infections in cattle and sheep as determined by positive antibody titers.

Species	No. tested	Number of Positive Titers *			
		Q Fever	RMsf	Tularemia	Brucella
Cattle	1,964	91	83	497	182
Sheep	196	0	5	0	0
Total	2,160	91	88	497	182

* Positive Titers: Q Fever and RMsf - 1:16 or greater
Tularemia - 1:40 or greater
Brucella abortus - 1:80 or greater

Plague, Pasteurella pestis:

Strain 9# 71 of P. pestis was isolated during 1959 from a pool of tissues of two Ord kangaroo rats, D. ordii, trapped May 7, seven miles north of Callao, Tooele County, Utah. This was in the same general area where 7E 755 was isolated by workers at Rocky Mountain Laboratory in May, 1957. The Callao area may be considered as a focus of enzootic sylvatic plague, since it is the only area where it has been found thus far in the survey. Repeated trapping of animals in this region has failed to yield additional evidence of infected animals. No noticeable change in the rodent population has been observed that would indicate an epizootic in the area.

The plague bacillus had been isolated from the tissues of the Ord kangaroo rat on only one previous occasion (1939), in Dona Ana County, New Mexico.[1] It was also found once in the ectoparasites (fleas) taken from Dipodomys species near Morton, Cochran County, Texas in 1947.[2] The existence of sylvatic plague in kangaroo rats is of interest because of the relative insusceptibility of the species to experimental intra- and subcutaneous injections, the LD_{50} being greater than 10^7 organisms. Susceptibility studies of the two isolates, 9F 71 and 7E 755, in selected native wild rodents and laboratory animals are summarized in Table 29.

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1 Eskey, C. R. and V. H. Haas. 1939. Plague in the western part of the United States. Pub. Hlth. Rept. 54:1467-1481.

2 Plague infection reported in the United States in 1946. Pub. Hlth. Rept. 62:1336-1340 (1947).

TABLE 29. *Pasteurella pestis* Virulence. A comparison of the LD_{50} of three strains of *Pasteurella pestis* in selected laboratory animals and wild rodents.

Species	Approximate number of organisms for an LD_{50}		
	Strain 7E 755	Strain 9E 71	Strain Alexander
Ord kangaroo rat *Dipodomys ordii*	$>10^5$*	$>10^6$*	$>10^7$*
Chisel-tooth kangaroo rat *D. microps*	$>10^5$	10^6	10^7*
Deer mouse *Peromyscus maniculatus*	10^4	10^5	10^4
Swiss albino mouse *Mus musculus*	$<10^2$	10^4	$<10^1$
Albino rat *Rattus rattus*	$<10^2$	10^4	—
Guinea pig *Cavia cobaya*	$<10^1$	10^3	10^3

* Largest number of bacteria injected.
All animals were injected subcutaneously in the inguinal region.

Tularemia, Pasteurella tularensis:

One strain of *P. tularensis* (9E 161) was isolated from a pool of 30 ticks taken from 11 black-tailed jack rabbits, *L. californicus*, Table 27. These animals were collected in the Dugway Valley area during November, 1959. This strain was found to be fully virulent for white mice, guinea pigs, albino rabbits and deer mice. Studies of this isolate and one (8F260) isolated in 1958 were made, and the resulting data are summarized in Table 30.

TABLE 30. *Pasteurella tularensis* Virulence. Approximate LD_{100} in selected laboratory animals and wild rodents of two strains of *Pasteurella tularensis* native to the Great Salt Lake Desert area.

Animal species	Approximate number/organisms constituting an LD_{100}	
	Strain 8F 260	Strain 9E 161
Deer mouse *Peromyscus maniculatus*	$<10^1$	$<10^1$
Swiss albino mouse *Mus musculus*	$<10^1$	$<10^1$
Guinea pig *Cavia cobaya*	$<10^1$	$<10^1$
Albino rabbit	$<10^1$	$<10^1$

P. tularensis agglutinating antibody at a titer of 1:90 was detected in the sera of two guinea pigs injected with a homogenate of ticks taken from *D. microps*, *D. ordii*, *C. leucurus*, and *P. longimembris*, collected in the Old River Bed area, Table 24. Using the same homogenate, repeated attempts to isolate the organism and to induce antibody formation in other guinea pigs, failed.

Agglutinating antibody titers of 1:40 or greater were detected in the sera of 11 animals (six species) trapped in five areas during 1959. The data are shown in Tables 22 and 23. This is the first time since the epizoological survey commenced in 1954 that sera from trapped native animals have shown antibodies against *P. tularensis*. Repeated attempts to isolate the organism from the tissues of the serologically positive animals have failed. The areas from which the animals were trapped were rather widespread, e.g., Johnson Pass is about 15 miles northwest of Lookout Pass and Vernon; Little Davis Mountain is 10 miles northwest, and Callao is 60 miles southwest. The animals involved were deer mice, mule deer, badger, chipmunk, and jack rabbit.

A total of 1,964 cattle and 196 sheep sera were tested for P. tularensis agglutinins. All sheep sera were negative. However, 308 (15.2%) of the cattle sera demonstrated titers of 1:40 and 89 (9.6%) demonstrated titers greater than 1:40, Table 23.

The tularemia tube agglutination test performed in this laboratory was compared with the test performed at Rocky Mountain Laboratory where much of this same type of work has been done. Each laboratory titered the same cattle sera and it was concluded that Epizoology Laboratory's test may be a two-fold serial dilution more sensitive.

Anthrax - Bacillus anthracis:

There was no evidence of B. anthracis in the tissues of the animals or the ectoparasites collected during 1959.

Brucellosis - Brucella sp.

One strain of Brucella neotomae (9F 201) was isolated from the tissues of a wood rat trapped in the South Wendover area in June, 1959, Tables 24 and 25. The strain was isolated by plating the tissues directly on Tryptose-phosphate agar. The injection of the tissues of this wood rat into several pairs of guinea pigs failed to induce a detectable infection. The strain was tentatively identified as Br. neotomae by the technique of Stcenner and Lackman.[1] This identification was later confirmed by workers at Rocky Mountain Laboratory. This is the second time the organism has been isolated by direct plating of tissues which, when injected into guinea pigs, failed to manifest evidence of infection.

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[1] Stoenner, H. G. and D. B. Lackman. 1957. A new species of Brucella isolated from the desert wood rat, Neotoma lepida Thomas. Am. J. Vet. Res. 28(69):947-951.

Previously, Br. neotomae isolations were made from animals collected in the Granite Mountain and Gold Hill areas. The isolation of the organism from the Wendover area could be indicative that this pathogen may be endemic in desert wood rats of the southwest section of the Great Salt Lake Desert.

Susceptibility studies of this organism in selected animals are reported in Table 31. It will be noted that the host, N. lepida, was only relatively susceptible to the organism when given intraperitoneally. Other routes of inoculation were not attempted.

Br. abortus agglutinating antibodies at a titer of 1:40 or greater were not found in the sera of the wild rodents or the sera of guinea pigs challenged with tissue and ectoparasite homogenates. These results are in accord with those of previous years.

All of the 196 sheep sera tested for Br. abortus agglutinins were negative, while 182 (9.2%) of the 1,965 cattle sera tested were positive at titers of 1:80 or greater, Table 29.

TABLE 31. Brucella neotomae Virulence. Susceptibility of selected animals to Brucella neotomae strain 9F 201.

Animal Species	Approximate number of organisms constituting an LD_{50}
Swiss albino mice Mus musculus	$<10^1$
Deer mice Peromyscus maniculatus	10^2
Guinea Pig Cavia cobaya	10^2
Desert wood rat Neotoma lepida	10^4

Data are based on studies using from 150 to 200 animals of each species. Infection was determined by production of agglutinating antibody, and the isolation of the organism from vital organs 21 days and more after challenge.